Response of Microalgal-Bacterial Consortia to Ocean Acidification and Crude Oil Pollution

Afiq Durrani Bin Mohd Fahmi

BSc. (Hons) Biotechnology

MSc. Marine Biodiversity and Biotechnology

Submitted for the Degree of Doctor of Philosophy

Heriot-Watt University

School of Engineering & Physical Sciences

July 2019

Abstract

Hydrocarbon-degrading bacteria play a crucial role in the recovery of marine systems in the event of an oil spill, and associate with various species of eukaryotic phytoplankton in the ocean. There is a paucity of knowledge to explain the relationship between these types of bacteria living associated with microalgae, and their collective response to oil spills in future ocean acidification (OA) conditions has not been studied to date. This thesis presents the first investigation that aims to understand the response of these organisms under future atmospheric CO_2 concentration (750ppm) and crude oil spills. Research was conducted on laboratory cultures of *Emiliania huxleyi* and natural community assemblage of subarctic surface seawater. Using high-throughput analysis of 16S rRNA sequencing, previously described key hydrocarbon degrading bacteria such as *Marinobacter, Alcanivorax*, and *Oleispira* were detected associated with microalgae. The response of bacterial community varied from being 1) negatively affected by OA and oil enrichment, 2) negatively affected by OA, but positively affected by oil enrichment under projected ocean acidification conditions, or 3) positively affected by OA and oil enrichment. *Marinobacter* and *Methylobacterium* were negatively affected by OA, which exacerbated the response of *E. huxleyi* to crude oil exposure. However, biodegradation of crude oil was not significantly affected. *Marinobacter* was also negatively impacted in the natural microbial community samples. *Polaribacter* was negatively affected in ocean acidification conditions and when exposed to crude oil enrichment. *Colwellia* was negatively impacted by OA but thrived during exposure to both crude oil and future ocean acidification conditions. *Sulfitobacter* and *Psychrobacter* was positively impacted by OA and oil pollution. Despite detection of hydrocarbonoclastic bacteria in natural community assemblage, their relative abundance in the bacterial community did not increase as expected after oil enrichment. Furthermore, no biodegradation of crude oil was detected in microcosms with natural microbial community. Highly abundant taxa in both spring and fall communities from the northeast Atlantic, such as *Colwellia* and members of families *Rhodobacteraceae* and *Halomonadaceae,* and the class *Gammaproteobacteria*, were persistent even when exposed to both stressors. In the event of an oil spill by the end of the century, OA favours selection of persistent and resilient bacteria that will outcompete hydrocarbonoclastic bacteria, thus delaying biodegradation and recovery from crude oil pollution in a future ocean.

Acknowledgements

To Dr. Sebastian Hennige and Dr. Tony Gutierrez: I deeply appreciate the opportunity and guidance you have given me, from the beginning of my PhD journey and hopefully for the rest of my career. I have learned many things from both of you through not just our discussions and meetings, but also from watching your day to day work.

This project was funded by University Malaysia Terengganu. Thank you very much to Prof. Ibrahim Komoo, Prof. Marinah, Mr. Zameri, and staff at UMT for believing in my dreams and supporting me throughout the entire project. Special mention to Prof. Hamish Mair for opening doors for me that have led me to where I am today.

To Dr. Steve Summers, and Dr. Fiona Murray: Thank you for training, supporting, and sharing your knowledge and experience with me. Both of you were always there to help me with questions and making sure I have what I needed to carry on with my work. There's not enough drinks and desserts in the world that I could give to repay your kindness. I owe special thanks to Prof. Murray Roberts and Dr. Lea-Anne for providing support and motivation in the Changing Oceans group.

To Robert, Margaret, Ellis, Stuart, Mary, Sean and Hugh: thank you for your hard work and goodwill that keeps my research, among many others running. You truly are the backbone of the university.

A very special mention to my Edinburgh family: Sally, Laura, Johanne, Danae, Alice, Feng Jia, Margaux, Ana R, Laurence E., Leagh, Laurence DC, Heather, David, Loris, Nina, Angelina, John, and Michi. Through lots of food and fun, you have made me feel at home in Edinburgh. You are my inspiration in pushing myself and keeping a healthy balance between work and life. Each and every one of you will always have a home in Malaysia.

To my friends: Ayesha, Nabil, Shah, Syaza, Hyqal, Farhan, Imran, Hariz, and Shufitri. Thank you for always being there for me and making sure I live my best life.

To my family: Thank you Mama and Ayah for the love and support you have given me. You have always been my strength and drive and I don't tell you often enough.

ACADEMIC REGISTRY
Research Thesis Submission

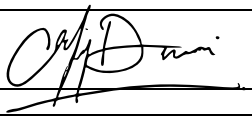
Name*:*	Afiq Durrani Bin Mohd Fahmi		
School:	School of Engineering and Physical Sciences		
Version: *(i.e. First, Resubmission, Final)*	Resubmission	Degree Sought:	Doctor of Philosophy

Declaration

In accordance with the appropriate regulations I hereby submit my thesis and I declare that:

1) the thesis embodies the results of my own work and has been composed by myself
2) where appropriate, I have made acknowledgement of the work of others and have made reference to work carried out in collaboration with other persons
3) the thesis is the correct version of the thesis for submission and is the same version as any electronic versions submitted*.
4) my thesis for the award referred to, deposited in the Heriot-Watt University Library, should be made available for loan or photocopying and be available via the Institutional Repository, subject to such conditions as the Librarian may require
5) I understand that as a student of the University I am required to abide by the Regulations of the University and to conform to its discipline.
6) I confirm that the thesis has been verified against plagiarism via an approved plagiarism detection application e.g. Turnitin.

* *Please note that it is the responsibility of the candidate to ensure that the correct version of the thesis is submitted.*

Signature of Candidate*:*		Date:	23/07/19

Submission

Submitted By *(name in capitals):*	
Signature of Individual Submitting:	
Date Submitted:	

For Completion in the Student Service Centre (SSC)

Received in the SSC by *(name in capitals):*			
Method of Submission *(Handed in to SSC; posted through internal/external mail):*			
E-thesis Submitted (mandatory for final theses)			
Signature:		Date:	

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

Human activities contribute to increased concentrations of greenhouse gasses (GHG) in the atmosphere that have consequential impacts on the environment. From 1970 to 2010, fossil fuel combustion contributed 78% of total GHG emission increase, and of that 76% was CO_2 (IPCC, 2014). Despite efforts in shifting to renewable energy, our dependency on fossil fuels increases pressure to drill oil deeper in the ocean and in higher latitudes, introducing more risks of oil pollution from drilling activity, tankers, and pipelines. This directly causes two problems to the marine environment.

Firstly, burning of fossil fuels releases GHG into the atmosphere, including CO_2. GHG not only affects climate, but also oceanic conditions. Atmospheric CO_2 is absorbed into the ocean and increasing levels of atmospheric CO_2 is directly correlated to ocean acidification. The ocean carbon sink has the capacity to remove 2.4 ± 0.5 GtC yr^{-1} of carbon from the atmosphere by photosynthetic activities in the marine environment and sequestration of carbon in the ocean sink (Le Quéré *et al.*, 2018).

Secondly, excavation, extraction and transport of crude oil increases the potential for oil spills to occur in the marine environment. The seawater-air interface is a region most vulnerable to ocean acidification and oil spills. Oil slick that forms on the seawater surface limits gas exchange and light penetration (Ozhan, Parsons and Bargu, 2014), thus inhibiting photosynthesis and "clogging" the carbon sink Susceptibility to crude oil vary in the microbial community (Ozhan, Parsons and Bargu, 2014), thus potentially reducing microalgal and bacterial diversity and limiting ecosystem function.

Roughly 50% of global primary production occurs in the open ocean via photosynthesis carried out by microalgae and cyanobacteria (Falkowski, 1994). The pelagic zone, or specifically the epipelagic zone, is a dynamic ecosystem that relies on autotrophic and heterotrophic organisms as a source of primary production and degradation i.e. nutrient cycling. Microalgal growth can be limited by nitrogen, that is made available by nitrogen fixing bacteria, and bacteria responsible for recycling and remineralisation of nitrogen via nitrification of ammonia to nitrite or nitrates (Voss *et al.*, 2013; Amin *et al.*, 2015). In exchange, bacterial community gets most of its carbon sources from microalgal metabolites. The intricate relationship between microalgae and bacteria in the water column, in addition to stressors of their physical environment is paramount to the health of the pelagic ecosystem. Projected increase in atmospheric CO_2 introduces rapid long-term changes to the marine environment that could be an additional stressor to microbial community response to oil pollution.

Ocean acidification is not only the decrease in pH of seawater, but also a process that causes shifts in seawater carbonate chemistry that affects buffering capacity (Hönisch

et al., 2012). Joint, Doney and Karl, (2010) proposed that marine microorganisms possess the flexibility to accommodate pH change and that there would be no catastrophic changes in marine biogeochemical processes that are driven by phytoplankton, bacteria, and archaea, but recognizes that calcification and photosynthesis could be affected. However, the question is how far can marine microorganisms be challenged with anthropogenic perturbations before detrimental effects to the marine ecosystem becomes apparent? At present, there is a lack in knowledge regarding the impacts of ocean acidification and oil pollution on the surface ocean microbial community. Cai *et al.*, (2011) reported that ocean acidification combined with eutrophication produces synergistic effects on bacterioplankton that differ from just ocean acidification alone. Therefore, it is of considerable importance that we address the following question:

What are the microbiological responses to
crude oil pollution in future ocean conditions?

1.1. Crude oil pollution

Oil introduced into the sea originates from four major sources or processes: 1) natural seeps, 2) petroleum extraction, 3) petroleum transportation, and 4) petroleum consumption. Due to its hydrophobic nature and slow biodegradability, crude oil compounds accumulate in sediments and in some marine organisms where they can elicit adverse effects on both human and environmental health (Nikolaou *et al.*, 2009). Spatial and temporal factors such as the scale of an oil spill, and the rate at which oil is introduced into the environment determines the fate of the oil and the bacterial response to it. Natural oil seeps represent around 47% of oil introduced in the marine environment, while 53% is derived from transportation of oil and other anthropogenic activities (Judd and Hovland, 2007; Duran and Cravo-Laureau, 2016).

Anthropogenic oil pollution can be introduced into the sea from transport tankers, pipelines, oil wells and drilling rigs (Xue et al., 2015). Although the incidences of oil spills from oil tankers that were more than 700 tonnes have decreased over the years (Figure 5), accidents do occur with dire consequences. The most recent oil spill reported in the media in April 2018 was from a ruptured undersea pipeline in Balikpapan, Indonesia, which resulted in an oil slick covering approximately 136 km^2 of coastal waters (Kahfi, 2018). Immediate environmental impacts reported included a stranded carcass of an Irrawady dolphin, and dead crabs that were found on the coast (Kahfi, 2018).

The scale of the Balik Papan spill was dwarfed by the infamous Deepwater Horizon (DWH) well blowout in the Gulf of Mexico in 2010. DWH released an estimated release

of 5 million barrels of oil was considered the worst oil spill in US history where impacts spanning from coastal environments to the deep sea (Mcnutt *et al.*, 2011). The scale of the spill impacted surface ocean, coastal and deep-sea ecosystems, in addition to subsurface plumes up to 1300 m deep in the Gulf of Mexico (Valentine *et al.*, 2014; Joye and Kleindienst, 2016). Studies on impacts of hydrocarbon plumes from DWH causing significant variation and loss of diversity when compared to non-plume communities and communities found on the surface (Redmond and Valentine, 2012). Due to the heterogenous compounds found in crude oil, it is important that we first understand its physical and chemical properties before understanding the fate of crude oil pollution on microbiological communities in the surface ocean.

1.1.1. Composition of crude oil

Crude oil comprises of saturated hydrocarbons, aromatic hydrocarbons, asphaltenes, and resins. Saturated hydrocarbons make up the bulk mass of crude oil, therefore their biodegradation is vital in removing crude oil from the environment (Head, Jones and Röling, 2006; Nikolaou *et al.*, 2009). The low-molecular-weight saturates, such as methane, ethane, propane, and the aromatics benzene, toluene, ethylbenzene and xylenes, are significantly soluble and highly susceptible to biodegradation (Prince et al., 2012).

Polyaromatic hydrocarbons (PAH) are considered priority pollutants that are persistent in the environment (Nikolaou *et al.*, 2009; Ma *et al.*, 2013). They compose of aromatic hydrocarbons and can be found in asphaltenes. Since they adsorb onto organic matter on the surface layer, they can be taken up into the food chain causing detrimental effects to biological systems (Head, Jones and Röling, 2006; Nikolaou *et al.*, 2009). PAH's are persistent in the environment due to their high molecular weight that influences the rate and extent of biodegradation of these chemicals (Marín-Spiotta *et al.*, 2014). Petrogenic PAHs enter the ocean through oil seeps and spills and are more bioavailable than pyrogenic PAHs which originate from atmospheric deposition and runoff from farmland, industrial effluents and urban storm waters (Duran and Cravo-Laureau, 2016).

1.1.2. Microalgal response to crude oil

Microalgae have been discovered to accumulate PAHs by directly sorbing these chemicals (Binark *et al.*, 2000), or they can indirectly influence the removal of PAHs by 1) enhancing air-water exchange through primary production, and 2) vertical sinking fluxes (Dachs *et al.*, 2002). Production of oxygen from photosynthesis carried out by

microalgae reduces the need for external aeration for the degradation of pollutants from crude oil such as PAH, phenolics and organic solvent especially when surface seawater-air interface is compromised by undissolved oil molecules (Muñoz and Guieysse, 2006). Crude oil toxicity affects structural and functional properties of phytoplankton, such as loss of cell permeability, reduced cell nuclei, loss of cell mobility, shrinkage of chloroplasts and pyrenoids, loss of CO_2 absorption, disruption of nuclei acid synthesis, reduced protein content, and damage to DNA and RNA from oxidative stress (Soto *et al.*, 1975; Sikkema *et al.*, 1995; Tukaj, Bohdanowicz and Aksmann, 1998; Koshikawa *et al.*, 2007; Parab *et al.*, 2008; González *et al.*, 2009). Therefore, the toxic effects of crude oil on phytoplankton communities directly disrupt the base of the marine food chain, and indirectly influences other trophic levels including bacteria communities through cascading effects (Hjorth, Forbes and Dahllöf, 2008). Recent studies have shown taxonomy, in addition to cell-size influence toxicity of crude oil on phytoplankton communities (Ozhan, Parsons and Bargu, 2014). Cyanobacteria have been found to be tolerant to high concentrations of oil, in addition to accumulating hydrocarbons in the inter-thylakoid spaces (Al-Hasan *et al.*, 2001). Smaller diatom species in a natural assemblage were shown thriving under acute toxicity of crude oil possibly due to liberation of predation (González *et al.*, 2009). On the contrary, larger sized phytoplankton have been reported to be more tolerant to crude oil due to their relatively lower cell surface to volume ratio (Sargian *et al.*, 2007; Echeveste, Agustí and Dachs, 2010). Diatoms were reported to be more sensitive to crude oil as the silica frustule adsorbs hydrocarbon thus retains toxicity of crude oil compounds and possibly hindering sexual reproduction (Siron *et al.*, 1991). Nutrient concentration and temperature were also identified as factors that affects sensitivity of phytoplankton to crude oil toxicity (Vargo, Hutchins and Almquist, 1982; Ozhan and Bargu, 2014). Therefore, it can be concluded that microalgal response varies between species and is further complicated by other factors introduced by the environment and community-level interactions.

1.1.3. Microalgal interactions with bacteria

Algal-bacterial interactions occur when bacteria and micro-algal cells are in close proximity to each other i.e. the phycosphere. The physical properties of molecules secreted by microalgae – i.e. the solubility and diffusibility of compounds – affects the spatial chemistry and, therefore, causes zonation in bacterial association (Seymour *et al.*, 2017). Microalgae supply carbon-rich compounds and amino acids that the bacteria utilise for growth, as well as in protecting them from desiccation and grazing (Amin *et*

al., 2009). For example, microalgae and cyanobacteria have the ability to produce alkane or alkene compounds from fatty acids (Sorigué *et al.*, 2016). Important hydrocarbon degrading bacteria have been isolated from phytoplankton cultures (Green *et al.*, 2006, 2015, Gutierrez *et al.*, 2012, 2013, 2014). Hydrocarbons produced by microalga could serve to sustain hydrocarbon-degrading communities, and their tolerance to temperature and influence in regulating climate could be significant in ensuring a continuity of hydrocarbon degrading bacteria in future ocean conditions (Shaw et al., 2003; Alvarez et al., 2009).

In addition to hydrocarbon production, microalgae have been shown to produce molecules that affect climate. Marine microalgal species like *Emiliana huxleyi* and *Skeletonoma costatum*, are amongst marine phototrophs that produce isoprene (2-methyl-1,3-butadiene), a biogenic volatile non-methane hydrocarbon that drives ozone production, increases residence time of gases that contribute to greenhouse effects and influences secondary organic aerosol formation (Shaw et al., 2003). Shaw et al., (2003) reported that the presence of heterotrophic bacteria in the phytoplankton culture did not affect isoprene production. However, no study has shown the effects of isoprene production by phytoplankton on the diversity of heterotrophic bacteria. Organosulfur molecules, such as taurine and dimethylsulfoniopropionate (DMSP), released by algae can be utilised by ammonium producing *Sulfitobacter sp.* (Seymour *et al.*, 2017).

Heterotrophic bacteria usually have a mutualistic relationship with microalgae. Cultures grown in axenic conditions, or free from bacterial associates, can negatively impact the physiology and growth of microalgae (Bolch, Subramanian and Green, 2011; Windler *et al.*, 2014). Most phytoplankton are vitamin B_1, B_7, and/or B_{12} auxotrophs, thus requiring mutualistic bacteria capable of synthesising these molecules (Croft *et al.*, 2005; Seymour *et al.*, 2017). In addition to vitamins, bacterial community associated to microalgae provide nutrients, such as nitrogen and iron, and growth factors to the microalgae (Bell, Lang and Mitchell, 1974; Croft *et al.*, 2005; Amin *et al.*, 2009). Nitrogen fixing bacteria, typically cyanobacteria, can provision microalgae with bioavailable nitrogen, which is especially important in oligotrophic conditions

Marinobacter spp., a key hydrocarbon degrader are commonly found associated with dinoflagellates and coccolithophores, but less so with diatoms (Amin *et al.*, 2009). Preferential selection of some clades of *Marinobacter* is linked directly to their ability to increase bioavailability of iron to their algal host. Prokaryotes utilise siderophores to bind iron, and *Marinobacter* produces a light sensitive siderophore, vibrioferrin, that has a relatively lower affinity to iron, which enables release of iron and increasing photolytic

activity in algal cells up to 10-20 times more than other photosensitive sideorophores (Amin *et al.*, 2009). Bacteria related to the *Roseobacter* clade that are commonly found associated with microalgae can secrete growth promoters and antibiotics that inhibit bacterial pathogens (Brinkhoff *et al.*, 2004; Geng and Belas, 2010; Thiel *et al.*, 2010; Seyedsayamdost, Carr, *et al.*, 2011). Relationship dynamics can often change from mutualistic to parasitism, as demonstrated by *P. gallaeciences* and host *Emiliania huxleyi*. Via this mutualistic partnership, the micro-algal host provides DMSP as a carbon source, as well as a surface for attachment for *P. gallaeciences*, whilst the bacterial symbiont produces growth stimulants, such as auxins. The metabolic switch to parasitism is triggered by the release of *p-coumaric acid* (pCA) by *E. huxleyi* during senescence, resulting in the production of roseobacticide by *P. gallaeciences* (Seyedsayamdost, Case, *et al.*, 2011).

This opportunistic parasitism, aptly referred to as 'the Jekyll and Hyde' interaction, is not unique to *P. gallaciences*, but was observed in *Dinoroseobacter shibae* associated to the dinoflagellate *Prorocentrum minimum* (Wang *et al.*, 2015), suggesting this is potentially a widespread strategy used by bacteria to maximize algal organic matter throughout the host life cycle. Extracellular protease secreted by *Kordia algicida* to disrupt cell walls of diatoms were reported (Paul and Pohnert, 2011). However, the diatom *Chaetoceros didymus* has been reported capable of developing a defence mechanism by secreting an algal protease effective against *K. algicida* protease (Paul and Pohnert, 2013). In order to secure a long-term nutrient source, bacteria don't necessarily kill micro-alga, but rather inhibit their growth instead. This is exemplified by members of the phylum Bacteroidetes that inhibit cell division of *Croceibacter atlanticus* after it successfully has attached to the algal surface, resulting in algal cell elongation and accumulation of plastids, an organelle found in microalgae (van Tol, Amin and Armbrust, 2017). Other prokaryotes have evolved to respond to other environmental cues, such as temperature, to elicit virulence. *Ruegeria* sp., a pathogen to macro-algae, extends its virulence to *E. huxleyi* at 25 ^{0}C, but exhibits mutualism or commensalism at 18 ^{0}C (Mayers *et al.*, 2016).

The strategies employed by heterotrophic bacteria in ensuring survival in the oligotrophic marine environment focuses on nutrient exchange between microalga, restricted by temporal and spatial variations of organic and inorganic compounds. Whether the bacteria are associated with the phycosphere, or living as endosymbionts inside microalgal cells, it is clear that these organisms aren't autonomous entities, but holobionts that contribute to the success of their micro-algal host in their environment

(Crump, Armbrust and Baross, 1999; Biegala *et al.*, 2002; Grossart *et al.*, 2005; Armbrust, 2009). Holobiont is described as a unit of biological organisation consisting of host and symbionts and their collective genomes which include a nuclear genome, organelles, and the microbiome (Bordenstein and Theis, 2015). Microbial holobionts are integral to the microbial loop, which is the equivalent of a microbial food web. The microbial loop is the transfer of carbon and energy from decomposition and remineralisation of inorganic and organic nutrients by heterotrophic bacteria (Pomeroy *et al.*, 2007). The remineralised organic matter is supplied back to primary producers to promote growth while bacteria enters the planktonic food chain via predation by flagellates which are then ingested by microzooplankton (Azam *et al.*, 1983). Therefore, the dynamic relationship of commensalism and predation between microbes that form the microbial loop optimises the environment through oxygen enrichment, waste removal, and conditioning of physicochemical parameters.

However, not all interactions are beneficial for microalgal cells due to the metabolic and behavioural diversity of heterotrophic bacteria and competition for resources. Growth inhibition is also an important strategy for bacteria competing for limiting nutrients with microalga, allowing higher bacterial growth rates that could outcompete algal growth (Ramanan *et al.*, 2016). Competition for nutrients does not necessarily elicit antagonism, or active hostility of bacteria with microalgae. Therefore, commensalism between bacteria that rely on microalga as a direct, or indirect carbon or sulphur source is plausible in the oligotrophic conditions of the pelagic zone. The diversity of heterotrophic bacterial species and their response to environmental conditions are therefore important in ensuring the survival of microalgal community, and the health of the environment. The microalgal-bacterial interaction and their interconnected functional roles may prove challenging when responding to stressors such as pollution.

1.1.4. Heterotrophic bacterial community response to oil spill

In natural systems, crude oil enrichment and toxicity reduces microbial diversity (Trevors, 1998). Microbial diversity is crucial for ecosystem resilience in response to environmental change (Von Canstein *et al.*, 2002). Microbial communities adapted to crude oil, such as those found in natural oil seeps, can degrade hydrocarbons much faster than microbial communities in pristine environments (Hazen et al., 2010; Joye, Teske, and Kostka, 2014). The heterogeneity of crude oil should theoretically support diversity of adapted bacterial communities in natural seeps; however, the selection pressure from hydrocarbon enrichment can negatively influence the diversity (Trevors, 1998;

LaMontagne *et al.*, 2004). Selective pressure from toxicity of hydrocarbon compounds on indigenous microbial communities supports and regulates other biota within the area by controlling the effects of oil on the environment (LaMontagne et al., 2004).

It was reported that 3.1% to 6.7% of total particulate PAH were removed in the euphotic zone (Adhikari, Maiti and Overton, 2015), while the rest will most likely reach deep sea-sediments (Gustafsson, Gschwend and Buesseler, 1997). Microbial community shifts due to oil spillage in the Gulf of Mexico were described for both the sea surface and subsurface within the oil plume during the Deepwater Horizon spill (Kimes *et al.*, 2014). Six months after the Gulf of Mexico disaster, community composition analysis of pelagic samples <800m deep were similar to the community prior to oil spill (Yang *et al.*, 2016). An important lesson learnt from studying the microbial community in the Gulf of Mexico was identifying important bacterial species and their roles within a hydrocarbon-degrading community, in addition to their limiting factors in performing those ecosystem roles.

1.1.5. Biodegradation of crude oil

Hydrocarbon degradation by microorganisms can take place under aerobic or anaerobic conditions. Aerobic degradation of crude oil is much more rapid and is initiated by mono- and dioxygenase enzymes produced by bacteria or fungi and uses oxygen as the terminal acceptor (Fritsche and Hofrichter, 2008). For the purpose of the background review in this thesis, focus will be given to marine bacterial biodegradation network in the pelagic zone that degrade hydrocarbons where aerobic conditions are prevalent. Hydrocarbon-degrading bacteria are mostly affiliated with the class Gammaproteobacteria, and to a lesser extent in the classes Alpha-, Beta-, and Delta-proteobacteria, as well as the phyla Actinomycetales (van Beilen and Funhoff, 2007). The degradation of hydrocarbons is a multiple step process involving competition and succession of a diverse bacterial assemblage that can be characterized as 1) obligate hydrocarbonoclastic (OHCB) bacteria, 2) generalists that can tolerate toxicity of hydrocarbon, or 3) putative opportunistic bacteria (McGenity *et al.*, 2012).

Blooms of marine OHCB rapidly degrade bioavailable saturated and aromatic hydrocarbons following an oil spill. Geographic distribution of OHCB were reported at various latitude/temperature, salinity, redox potential, among other physicochemical conditions (Figure 1.1;Yakimov, Timmis and Golyshin, 2007). Marine OHCB are specialists in utilizing hydrocarbons as a sole source of carbon and energy, unlike their terrestrial hydrocarbon degrading counterparts that are more versatile in their metabolic capabilities (Yakimov et al., 2007).

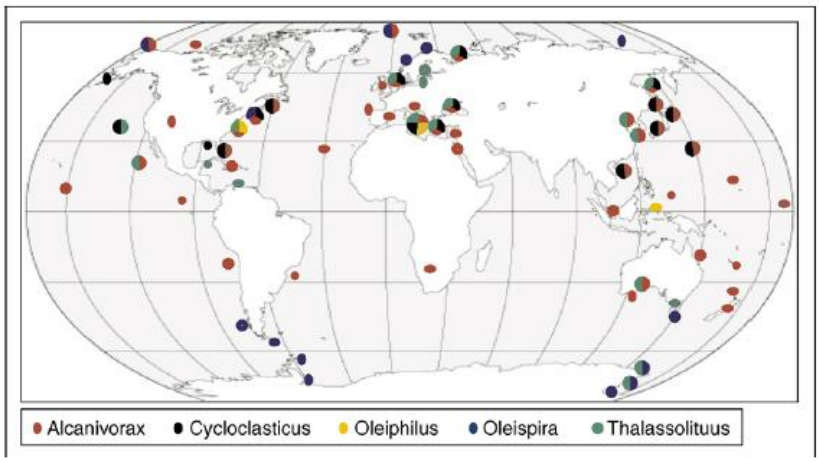


Figure 1.1 Geographic distribution of OHCB based on isolates and detection of 16S rRNA gene sequences (Yakimov, Timmis and Golyshin, 2007).

Bacteria within the genus *Alcanivorax* and *Cycloclasticus* have been found to be strictly OHCB or metabolically diverse, however bacteria in the genus *Oleiphilus, Oleispira*, and *Thalassolituus* are strictly OHCB (Yakimov, Timmis and Golyshin, 2007). Marine OHCB display features typical of oligotrophic bacteria and occupy a unique trophic niche among heterotrophic bacteria that mediate the removal of toxic oil pollutants that may not be substrates for most bacteria from the marine environment (Yakimov, Timmis and Golyshin, 2007). *Alcanivorax* were primary responders to oil enrichment in a previously unpolluted seawater, resulting in blooms that make up 90% of the microbial community succeeded by *Cycloclasticus* spp. (Yakimov, Timmis and Golyshin, 2007). Microcosm studies of oil enrichment using Arctic seawater maintained at 4^0C resulted in blooms of *Oleispira* (Yakimov *et al.*, 2003; Coulon *et al.*, 2007).

The diversity and geographic distribution suggest ability to carry out functional role of hydrocarbon degradation under most environmental conditions. However, little is known about the rapidly changing seawater carbonate chemistry from increasing levels of atmospheric CO_2 and how that may affect microbial community structure and function.

1.2. Changing Ocean

1.2.1. Ocean acidification

Ocean acidification (OA) is the shift in seawater chemistry resulting from the increase of CO_2 influx into the marine environment. Although it is notably measured by the decrease in the concentration of hydrogen ion in a solution measured on a logarithmic scale (pH), the equilibrium of carbonate chemistry in seawater is affected by OA. The Climate Change 2014 Synthesis Report clearly states with high confidence that oceanic uptake of CO_2 from the atmosphere has reduced seawater pH by 0.1, correlating to an increase in hydrogen ion concentration by 26%, and is currently the fastest rate of ocean acidification in history (IPCC, 2014). Seawater chemistry of dissolved inorganic carbon exists in equilibria as shown below.

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{2-}\ H^+$$

When carbon dioxide dissolves in seawater, carbonic acid (H_2CO_3) is produced, albeit at a slower rate than the ionisation of carbonate acid to bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions. Total alkalinity (A_T) is the mass conservation expression for hydrogen ion relative to a chosen zero value (Riebesell *et al.*, 2011). A_T is expected to remain constant under OA conditions as the charge balance in seawater will remain the same when positive ions generated from increase of CO_2 is balanced by negative ions. The degree of CO_2 saturation in seawater samples is determined by the partial pressure of CO_2 in the air and in equilibrium with seawater (pCO_2). As dissolution of CO_2 concentration in the atmosphere (pCO_2) increases, total dissolved inorganic carbon species (C_T) or the concentration of carbon species comprised of CO_2 (aq), bicarbonate (HCO_3^-), and carbonate ions (CO_3^{2-}) in seawater increases; carbonate ions will decrease as increase in hydrogen ions will sequester carbonate ions and form bicarbonate ions.

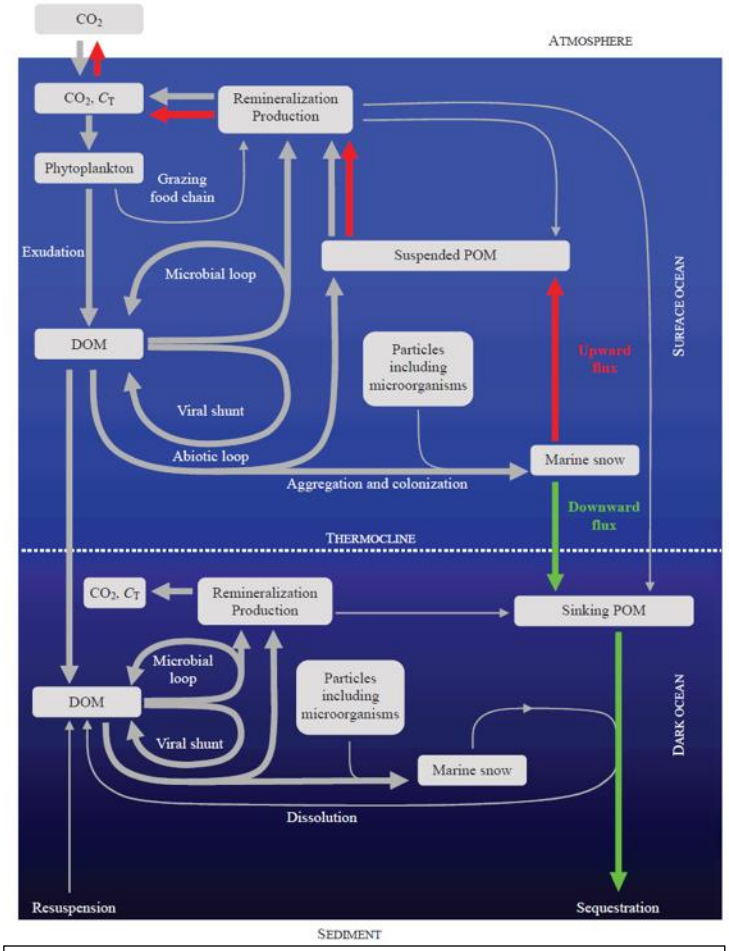


Figure 1.2. Potential effects of OA on vertical cycling of organic nutrients from microbial. Thick lines shows high probability of OA affected processes while thin lines shows OA less likely to affect processes. Red arrows indicate processes enhanced by OA resulting in aggregation and remineralisation of organic matter, while green arrows indicate enhanced vertical transport of organic matter in to deeper ocean (Weinbauer, Mari and Gattuso, 2011).

The change in physicochemical parameters affects nutrient cycling and remineralization of compounds in the marine environment, as illustrated in figure 1.2. OA enhances remineralization of dissolved organic matter and promotes formation of transparent exopolymer particle (TEP), as a result of increased buoyancy of aggregates at lower pH (Mari, 2008; Weinbauer et al., 2011). An upward and downward flux of POM leads to more organic matter in the euphotic zone, as well as particle sinking to the dark ocean. Both processes are not mutually exclusive, and the net effect is still unknown (Weinbauer, Mari and Gattuso, 2011). Increased TEP production under high CO_2 conditions and lower pH conditions in surface water leads to stratification that allows for vertical stability of nutrients since aggregates become less buoyant under lower pH (Weinbauer, Mari and Gattuso, 2011). When combined with stagnant water, where residence time of water is long, these conditions promote blooms of cyanobacteria, but eukaryotic microalgae has the advantage during conditions involving more water mixing, as they are relatively faster growing than cyanobacteria (Weinbauer and Wenderoth, 2008; Paerl and Paul, 2012).

1.2.2. Microalgal response to OA

Extensive studies on the response of prokaryotic cyanobacteria, as well as eukaryotic microalgae such as diatoms, dinoflagellates, and coccolithophores to OA conditions have shown differences in community level, as well as species-specific variation. Microalgal growth rates are regulated on factors such as nutrient acquisition, cell size, and photoprotection (Mackey *et al.*, 2015). Under OA conditions, these factors are directly and indirectly affected due to the shift in equilibrium of seawater carbonate chemistry. These differences can be attributed to regulation of carbon concentrating mechanism (CCM) for photosynthesis. CCM enables saturation of CO_2 within cells of microalgae even when CO_2 levels are low. Therefore, an increase in CO_2, a substrate required for photosynthesis, does not directly increase photosynthetic rates of microalgae up to a certain threshold unlike other substrates that conform to Monod's kinetic of growth substrates. When compared to ambient CO_2 levels, reduction of energy demand required for CCM were calculated to reduce by ~20% when CO_2 concentration was doubled (Hopkinson *et al.*, 2011). The bulk of energy demand for CCM is from the active transport of HCO_3^- from cytoplasm to carboxysomes where it is converted to CO2 by carbonic anhydrase that can be recycled back to HCO_3^- in the cytoplasm (Mackey *et al.*, 2015). CO_2 leaks into cytoplasm and is passively diffused between the cell and the surrounding environment therefore, under high pCO_2 levels energy demand is directly proportionate to gradient of CO_2 from seawater to site of fixation (Mackey *et al.*, 2015).

Cyanobacteria are the oldest known prokaryotes dating back to 3.5 billion years ago, thus are expected to be highly adaptable to changing oceans (Schopf, 2000; Paerl and Paul, 2012). In laboratory conditions, maximal photosynthetic rates of dominant picocyanobacterial *Prochlorococcus* and *Synechococcus* were not affected even when *Synechococcus* pigments shows higher light harvesting efficiency and lower light saturation constant under elevated pCO_2 (750 ppm) conditions (Fu *et al.*, 2007). Interestingly, field studies show increased ratio of *Synechococcus* to *Prochlorococcus* when pCO_2 levels are higher with no effect on cell size and pigment content (Lomas *et al.*, 2012). Only when OA stressor was combined with increasing temperatures (+ 4 ^oC), photosynthetic rates of *Synechococcus* increased four-folds, followed by increase in growth rate by two-folds suggesting an advantage of *Synechococcus* in OA conditions that are regulated by temperature-dependent regulation of enzymes (Mackey *et al.*, 2013).

Diazotrophic cyanobacteria or nitrogen fixing cyanobacteria from the genus *Trichodesmium* were largely found to be positively influenced by higher CO_2 conditions.

Growth rate, biomass production, C acquisition, and/or N_2 fixation were all stimulated under high CO_2 conditions (Barcelos e Ramos *et al.*, 2007; Hutchins *et al.*, 2007, 2013; Levitan *et al.*, 2007; Kranz *et al.*, 2009, 2010; Levitan, Kranz, *et al.*, 2010; Levitan, Sudhaus, *et al.*, 2010; Garcia *et al.*, 2011; Spungin, Berman-Frank and Levitan, 2014) however N_2 fixation and growth under high CO_2 conditions were negatively affected under Fe limitation (Shi *et al.*, 2012). Change in pH levels were found to negatively affect nitrogenase activity (Shi *et al.*, 2012). In addition, higher CO_2 did not increase photosynthetic rates of *Trichodesmium* but alleviates energy demand of CCM (Levitan *et al.*, 2007; Kranz *et al.*, 2010).

As for eukaryotic microalgae, different CCM strategies involving inorganic C transporters, internal and external carbonic anhydrase, in addition to presence/absence of pyrenoid, a microcompartment for rubisco located within chloroplast further complicates and diversifies response of eukaryotic microalgae to ocean acidification (Mackey *et al.*, 2015). The model species for diatoms, *Phaeodactylum tricornutum* responds to high CO_2 conditions by increasing rates of photosynthetic electron transport, with little to no change in growth rates (5-13%) or C fixation rates (Burkhardt *et al.*, 2001; Wu, Gao and Riebesell, 2010; Li, Xu and Gao, 2014). *P. tricornatum* displays low cellular affinity for inorganic C since no stimulation in photosynthesis was observed even when exposed to 5,000 ppm of CO_2 (Matsuda, Hara and Colman, 2001). This low affinity for inorganic C is likely a strategy to allocate energy to fuel other metabolic processes that may lead to subsequent loss of photoprotection (Burkhardt *et al.*, 2001; Wu, Gao and Riebesell, 2010; Li, Xu and Gao, 2014). Uptake of HCO_3^- and CO_2 proportions by the diatom *Thalassiosira pseudonana* is comparable to *P. tricornatum*, however *T. pseudonana* shows relatively lower CA activity accompanied by higher light saturation for photosynthesis due to increased maximum photosynthetic rate (P_{max}) and PE curve half saturation constant (E_k) (Sobrino, Ward and Neale, 2008). Considering diatoms are responsible for 40% of oceanic primary production in different and often extreme habitats (Nelson *et al.*, 1995), the observed species-specific responses of laboratory strains of diatoms to OA conditions previously mentioned emphasizes the need for understanding the effects of OA on diatoms and the impending impact on global carbon cycle.

Diatoms in the upper water column are capable of forming intense blooms due to their efficient CCM and diverse mode of C acquisition that allows for energy allocation for metabolic processes and growth (Mackey *et al.*, 2015). Field experiments and mixed culture laboratory experiments show shifts to diatom dominated communities under OA conditions (Tortell *et al.*, 2002, 2008; Hoppe *et al.*, 2013). In a study that shows increase

in abundance of diatoms compared to *Phaeocystis*, total biomass and primary productivity was not affected by increased CO_2 concentrations (Tortell *et al.*, 2002). Whilst a separate study states that under high CO_2 concentrations, selection of larger chain-forming diatoms led to higher productivity thus enhancing C export by faster sinking of cells (Tortell *et al.*, 2008). Larger sized diatoms conserve more energy than smaller cells due to smaller surface area to volume ratio that allows relatively lesser reliance of passive transport of CO_2 (Wu *et al.*, 2012; Mackey *et al.*, 2015). Although size can play a role in species selection under high CO_2 conditions, other studies have shown contradicting results where picoplankton outcompeted diatoms (Riebesell *et al.*, 2013). Picoplankton also showed increased net photosynthesis and carbon consumption in a mixed community dominated by coccolithophores and diatoms (Riebesell, 2004).

Termination of coccolithophore blooms may have different biogeochemical consequences on the ratio of inorganic carbon to organic carbon in seawater. Increased photosynthesis by coccolithophores due to rise in CO_2 levels have shown to reduce the ratio of inorganic C to organic C in coccolithophores, thus affecting C export from the atmosphere to the deeper ocean (Riebesell *et al.*, 2000; Zondervan, Rost and Riebesell, 2002; Langer *et al.*, 2006, 2009; Feng *et al.*, 2008; Shi, Xu and Morel, 2009; De Bodt *et al.*, 2010; Müller, Schulz and Riebesell, 2010; Fiorini, Middelburg and Gattuso, 2011; Lefebvre *et al.*, 2012; Rokitta and Rost, 2012). However, contradicting results regarding the impact of elevated CO_2 on the maximum photosynthetic rates between strains of the cosmopolitan *E. huxleyi* have become apparent (Feng *et al.*, 2008; Rokitta and Rost, 2012). Nutrient concentration have been shown to affect response of *E. huxleyi* under high CO_2 conditions as growth was enhanced with nitrate enrichment but depressed when grown with ammonia and nitrate enrichment (Lefebvre *et al.*, 2012). Photosynthesis is a net sink of CO_2 whilst calcification is a net source for CO_2. Since coccolithophores only produce calcium carbonate shells during the diploid stage of their life cycle, termination of bloom during this phase by predation from grazers increases sinking rates and export of C into deeper oceans (Mackey *et al.*, 2015).

Fossil records have shown prominence of heavily calcified cells during eras with lower levels of CO_2 that suggests calcification will be greatly affected under projected rise in CO_2 levels (Beaufort *et al.*, 2011). Orr *et al.*, (2005) predicted undersaturation of calcite in high latitude regions thus favouring dissolution of calcite due to increase in CO_2 and consequential reduction in pH levels by the year 2100. Majority of coccolithophores have been observed to reduce levels of calcification when grown under elevated CO_2, while certain strains from the same species have shown to increase calcification under

elevated CO_2 (Iglesias-Rodriguez *et al.*, 2008; Shi, Xu and Morel, 2009). Observations of degraded or atypical coccoliths under elevated CO_2 conditions suggest OA conditions induce stress on coccolithophores (Riebesell *et al.*, 2000; Langer *et al.*, 2006; Feng *et al.*, 2008; Iglesias-Rodriguez *et al.*, 2008; Barcelos e Ramos, Müller and Riebesell, 2010; De Bodt *et al.*, 2010; Müller, Schulz and Riebesell, 2010; Langer and Bode, 2011; Müller *et al.*, 2012). Higher production of dimethylsulfoniopropionate (DMSP) by coccolithophores were seen when grown under OA conditions (Spielmeyer and Pohnert, 2012). DMSP is the precursor to dimethylsulfide (DMS) a climate influencing compound that promotes cloud formation, and an important solute for marine bacteria that influences availability of metals ions (Spielmeyer and Pohnert, 2012; Dey and Brummett, 2018).

Dinoflagellates are a metabolically versatile group of motile protists that consists of phototrophs, mixotrophs capable of switching to phototrophs, and obligate heterotrophs that can be predators or parasite to other organisms (Mackey *et al.*, 2015). Phototrophic dinoflagellates have CCM with rubisco similar to anaerobic non-sulphur purple bacteria but are distinctly different from other phototrophs (Whitney, Shaw and Yellowlees, 1995). When exposed to OA conditions, photorespiration rates of dinoflagellates differ between species and is attributed to their diversified CCM between species (Mackey *et al.*, 2015). Dinoflagellate CCM may vary between species in the following ways: 1) rubisco with different affinity for CO_2 (Whitney, Shaw and Yellowlees, 1995), 2) utilising HCO_3^-, 3) absence/presence of external carbonic anhydrase (Dason, Emma Huertas and Colman, 2004), 4) absence/presence of pyrenoid, and 5) phosphoglycolate phosphatase enzyme (Ratti, Giordano and Morse, 2007). However, dinoflagellate blooms are often associated with toxin production. Elevated CO_2 and nutrient-limited conditions increased growth rate of the dinoflagellate *Karlodinium veneficum* in addition to increased production of toxin when grown under high CO_2 and P limited conditions (Fu *et al.*, 2010). Another bloom forming dinoflagellate *Alexandrium catanella* increased production of saxitoxin under increased CO_2 conditions regardless of nutrient limitation or elevated temperature (Tatters *et al.*, 2013).

In conclusion, response of microalgae to high CO_2 conditions vary between species due to diversity of CCM, and can be exacerbated by other factors like cell size (Wu *et al.*, 2012), temperature, light irradiance (Fu *et al.*, 2007), and nutrient limitation (Hoppe *et al.*, 2013). Strategies implemented by microalgae that have been proven successful to climate stressors include 1) resting cells, or cysts, 2) photoprotective capsule or sheath, 3) photoprotective pigments, 4) vertical and horizontal motility via gliding and buoyancy (Whitton and Potts, 2000; Reynolds, C., 2006), 5) physiological adaptation to nutrient

depletion (Gallon, 2006; Reynolds, C., 2006; Cottingham *et al.*, 2015), 6) produce metabolites that improve surrounding environment (Paerl and Millie, 1996; Paerl and Otten, 2013), and 7) mutualistic associations (Paerl, H., 1982). Important substrates for microalgal growth originating from inorganic and organic compounds are made bioavailable when fed into the microbial loop that consists of metabolically diverse heterotrophic bacteria, thus highlighting the importance of the bacterioplankton community found on the surface water in predicting the robustness of an ecosystem to impacts of OA.

1.2.3. Heterotrophic bacteria in surface ocean response to ocean acidification

Nutrient cycling of heterotrophic bacteria that are free-swimming or directly associated to microalgae varies in response to OA and climate change, resulting in changes in taxonomic levels of bacterial communities (Bretter *et al.*, 2016). These variations can be attributed to their metabolic capabilities and can be identified to two types of strategies for adaptation: 1) r-strategists, and 2) K-strategists. The r-strategists have high growth rates in nutrient rich environments, adapted to rapid changes in types and concentrations of nutrients (Egli, 1995), whereas K-strategists show low growth rates at low nutrient conditions best adapted to stable oligotrophic conditions (Egli, 2010). In the pelagic environment, it can be assumed that r-strategists are bacterial communities found in the phycosphere or attached to phytoplankton host where their nutrient and environmental control depends on host interactions. K-strategists are, therefore, free-living bacteria scavenging for nutrients and not closely associated to microalgae. Remineralisation of nitrogen, phosphorus, iron, and sulphur by prokaryotes contributes to nutrient-rich organic matrix that can be considered a physical refuge for microorganisms (Alldredge and Cohen, 1987; Weinbauer, Mari and Gattuso, 2011) . Other nutrient refuges that may ensure survival of heterotrophic bacteria in future ocean conditions includes dissolved polymers released by heterotrophic bacteria that may lead to self-aggregation of exopolymers (Passow, 2002).

Besides metabolic versatility, oxidative stress may also play a role in the taxonomic selection of bacteria in a changing ocean, specifically in oxic environments of the pelagic zone. The oxygen content in the environment affects intracellular production of ROS, as oxygen can readily pass through the membrane of a microorganism (Bretter *et al.*, 2016). In coping with reactive oxygen species (ROS), heterotrophic bacteria have adapted three strategies: 1) avoidance of generating ROS and reactive nitrogen species, 2) antioxidants, such as pigments that scavenge for ROS, and 3) repair and replacement of mechanisms damaged by ROS (Bretter *et al.*, 2016). Extracellular exportation of

oxygen avoids ROS production while producing enzymes without reactive metals and co-factors, like copper and iron, decreases ROS production (Medigue *et al.*, 2005). Repair of cellular mechanisms highly depend on nutrient availability, and thus oligotrophic conditions generally do not favour reparation or replacement, as hypothesized by Swan *et al.*, (2013).

Therefore, environmental processes such as primary productivity, organic matter degradation, and extracellular enzymatic activity by heterotrophic bacteria are affected by OA conditions resulting in change of microbial community dynamics (Das and Mangwani, 2015). Change in bacterial community dynamics from OA over longer timescale may introduce prolonged impacts on the ecosystem. This is vital when confronted with additional stressors such as crude oil pollution. The sudden introduction of a mixture of complex hydrocarbon compounds during an oil spill under OA conditions may challenge the robustness of microbial community in the surface ocean.

1.2.4. Changing ocean impacts on hydrocarbon degrading microbial communities

Based on different metabolic strategies and ROS-coping mechanisms, the entry of hydrocarbons into the marine environment may have an effect on microbial communities and in their response and biodegradation of hydrocarbons. Host-microbiota interactions may also play an important role in hydrocarbon degradation in the epipelagic zone. Microalgae and their associated bacteria can influence the microbial response to hydrocarbon pollution and may be more adept at removing hydrocarbons compared to free-living communities (Thompson *et al.*, 2017). Adsorption, absorption and vertical transport of pollutants by microalgae is important for pollutant uptake (Dachs et al., 2002). In the epipelagic zone, microalgae are likely to influence pH and nutrient availability that can in turn influence bacterial assemblages and their response to hydrocarbon pollution.

Studies on hydrocarbon-degrading microbial communities in the context of a changing ocean have focused on bacteria in marine sediments that were highly influenced by temperature increase (Coelho *et al.*, 2014, 2016; Bargiela *et al.*, 2015). Eight oil polluted sites were compared, and temperature was identified as the dominant driver of bacterial community species distribution, compared to environmental constraints, geography, O_2 concentration, and crude oil inputs (Bargiela *et al.*, 2015). Rich diversity of prokaryotes at sites with lower temperature showed overlaps and complementary functional roles for hydrocarbon biodegradation, while sites with higher temperature were dominated by populations of high catabolic diversity (Bargiela *et al.*, 2015).

Oil contamination in combination with modest OA conditions also showed changes in structure of bacterial communities, the consequence highlights change in oil chemistry resulting in increased toxicity to benthic communities (Coelho *et al.*, 2015). The increased toxicity was hypothesized to be in response to either 1) the direct effect of reduced pH on oil chemistry, 2) indirect reduction in soil quality from the decrease in abundance of *Desulfobacterales*, or 3) toxic compounds produced by microbial community adapted to OA and oil contamination (Coelho *et al.*, 2015).

Currently, limited studies on combined effects of OA with other environmental stressors have shown potential in increasing effects of bioavailability and biotoxicity of heavy metals and increasing bioavailability of nutrients (Zeng, Chen and Zhuang, 2015). No available studies have indicated the effects of OA on hydrocarbon degrading microbial communities in the marine water column, specifically the euphotic zone. Due to the short generation time of microbes, and functional role in biogeochemical cycles of the ocean, bacterial communities associated with microalgae are excellent characteristics to model climate change scenarios on the detoxification processes in the ocean (Coelho *et al.*, 2013).

1.3. Thesis outline

This thesis aims to determine the response of open ocean microbial community, specifically microalgae and their associated bacterial community to ocean acidification and crude oil pollution. To achieve this, the microcosm approach was used for all the experiments involving simulation of ocean acidification and crude oil pollution impacts on microalgal-bacterial consortia carried out within the project. This allows for a controlled environment and a transportable benchtop microcosm setup allowed for continuous aeration of ambient/mixed gasses, and crude oil enrichment/extraction for laboratory and on-board experiments that can easily be interchangeable to suit the purpose and location of experiments.

In Chapter 2, the various methods used throughout this thesis, such as culture-dependent techniques, next generation sequencing, and various chemical analyses (i.e. seawater chemistry and hydrocarbon analysis) were elucidated. Chapter 3 looks at the impacts of OA and oil pollution on a monoculture of the non-axenic coccolithophore *Emiliania huxleyi*. Chapter 4 and Chapter 5 looks at the response of a natural bacterioplankton community from a subarctic region in the northeast Atlantic that was sampled during spring and fall of 2017, respectively. A conclusion and summary of the findings in this thesis is presented in Chapter 6 and with a view to future work.

Chapter 2: Experimental Design and Methodology

2.1. Introduction

For the purpose of understanding microbiological response to future ocean conditions and crude oil pollution, a batch culture microcosm approach coupled with molecular techniques was chosen to characterize the bacterial species diversity and their ecosystem function. Microcosms utilize a limited volume of medium, hence nutrients to allow observation of growth patterns and metabolism throughout the experiment (Fogg and Thake, 1987). By maintaining a simplified system under high-degree of controlled conditions including CO_2 concentrations, temperature, light cycles and light intensity (Roeselers *et al.*, 2006). Factors such as high degree of environmental control, ease of replication of treatments, and containment of toxicant in a closed system in a microcosm setup (Jessup *et al.*, 2004; Coelho *et al.*, 2013) allows for safe and relevant observations of the dynamic interaction between microalga host and microbiota in the euphotic zone in response to independent or interactive treatments; elevated CO_2 and crude oil enrichment. The limitations of spatial and temporal scales due to the size of culturing vessel of the microcosm is minimized considering the small size and short generation time of microorganisms, thus enables capturing of essential dynamic interaction without fully reproducing nature in a laboratory model system (Jessup *et al.*, 2004).

Phytoplankton and associated bacteria were exposed to independent or synergistic effects of OA and crude oil enrichment. Treatments kept constant between all experiments were 1) aeration with ambient or elevated CO_2, and 2) enrichment of crude oil for biological and chemical analysis. Triplicates were carried out for each treatment. Chemical analysis of hydrocarbon compounds potentially degraded by bacteria associated to phytoplankton required a separate set of triplicates. Microcosms with crude oil for chemical analysis remained untouched for the entire experiment from the moment crude oil was introduced to prevent loss of hydrocarbon through sampling. Furthermore, a control for hydrocarbon analysis where the culture was acid-killed with a final concentration of 3% phosphoric acid prior to oil enrichment was done to remove bias of hydrocarbon compounds produced by phytoplankton, or adhesion of hydrocarbon compounds to surface of microorganisms that cannot be separated during liquid-phase separation of crude oil from culturing media. To summarize, each CO_2 treatment (ambient or elevated) is exposed to 12 microcosms that consists of triplicates of 1) positive control of inoculant growth without oil, 2) acid-killed inoculant culture with oil enrichment, 3) inoculants with crude oil enrichment for biological sampling, and 4) inoculants with crude oil enrichment for chemical analysis.

2.2. Materials

The microcosm setup consists of a versatile framework for simulating OA scenarios in epipelagic zone in a closed system that allows use of toxicants such as crude oil. Factors such as safety, reproducibility, minimal space, cost efficiency, ease of transport and assembly for culturing in laboratory conditions or on-board a research vessel informed the design of microcosm and experiments. Materials chosen were affordable and easily available from online suppliers, thus enabling replication of the experiments. Fundamental aspects of biological activity in marine ecosystems, such as temperature, photoperiod, and light intensity (Coelho *et al.*, 2013) could be easily established, controlled and monitored, while maintaining a stable supply of predetermined atmospheric CO_2 to manipulate pH and carbonate chemistry through gas mixing. The gas mixing system employed here enables high-sensitivity CO_2 manipulation and can be easily assembled within a small space.

2.2.1. Microcosm design

Microcosms were constructed using 500ml Youtility bottles (Duran) coupled with GL45 4-inlet bottle caps (Duran; Figure 2.1). Youtility bottles are smaller in diameter compared to other 500-ml laboratory bottles, thus reducing surface area while maximizing vertical distribution of culturing media in microcosms. Two of the four-inlet bottle caps were used for channeling air into and out of the microcosm while the remaining two inlets were sealed. Figure 2.1 illustrates the assembly of the microcosm and its components. Each microcosm was fitted with a 0.22 μm inline air filter attached to the inlet of the GL45 bottle cap and another air filter fitted on the tubing carrying the outflow of air to prevent microbial contamination. A glass Pasteur pipette connected to the inside of the GL45 cap channels the inflow of air into the seawater thus aerates and agitates the seawater by bubbling. Due to the characteristics of crude oil, i.e. strong adsorption to plastic, the use of plastic hardware in setting up these experiments was avoided. Glass Pasteur pipettes, for example, were used to provide aeration and doubles as a channel for sampling thus prevents plastic tips from touching crude oil.

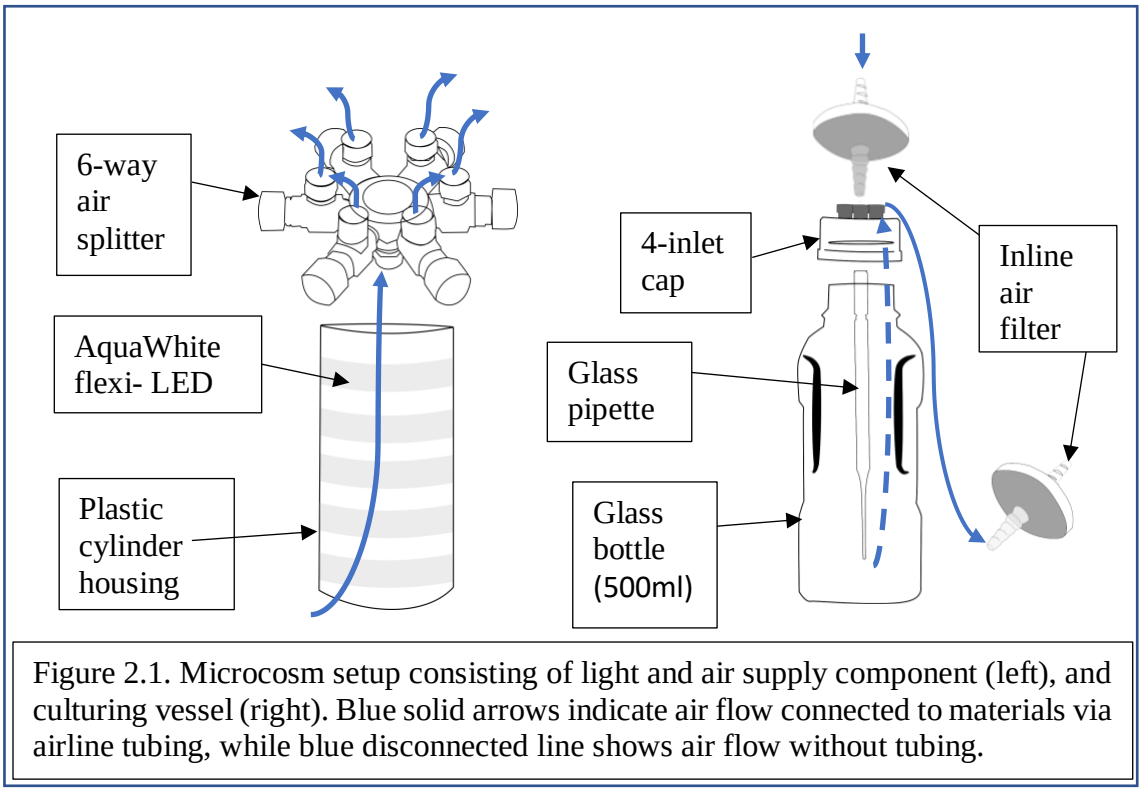


Figure 2.1. Microcosm setup consisting of light and air supply component (left), and culturing vessel (right). Blue solid arrows indicate air flow connected to materials via airline tubing, while blue disconnected line shows air flow without tubing.

2.2.2. Lighting system

The microcosms were arranged at equidistance from a central, vertical light source (Figure 2.1) to ensure each microcosm received equal light intensity. This arrangement avoids self-shadowing of microalga from exponential reduction of light-intensity with increasing path length, as per Beer's law (Myers, 1953), and shadowing from bottle caps and airline connections that occurs when horizontal lighting is supplied from above, such as from the top of the incubator. Furthermore, arranging the microcosms around the cylindrical light source uses space more effectively than a setup where microcosms are arranged parallel to a vertical light panel. AquaWhite flexi-LED (Aquaray) strips were used to construct the light cylinders by wrapping them around a plastic tube for a centralized vertical light source for each microcosm setup. Neutral density filters were used to reduce the light intensity to 58 umol m^{-2} s^{-1}, and diurnal cycles were maintained at a 16:8 h light:dark cycle. The AquaWhite flexi-led is also waterproof, thus allowing partial submergence in the water bath used during on-board experiment.

2.2.3. Gas mixing

Aeration was supplied at two atmospheric CO_2 conditions for all experiments; ambient (400ppm) and elevated (750ppm). Ambient air was supplied from air outside the building of the laboratory or the air outside of the laboratory on the research vessel using

an air pump. Microcosms were aerated with ambient air containing 400 ppm of atmospheric CO_2, or using elevated levels of atmospheric CO_2 to 750 ppm. This higher CO_2 concentration (750 ppm) is the midway value of SRES scenario A1B and A2 and is used as the target for future elevated atmospheric CO_2 conditions predicted by the year 2100, as recommended by the European Commission's guide to best practices for ocean acidification research and data reporting when comparing two atmospheric CO_2 conditions (Agostini, Riebesell and Gattuso, 2011). This elevated CO_2 concentration is the moderate projection influenced by the ongoing efforts to curb fossil fuel CO_2 emissions, recent emission records, and climate modelling that have been agreed upon and is widely used amongst researchers studying ocean acidification (Nakicenovic and Swart, 2000; Plattner *et al.*, 2001).

Elevated CO_2 was achieved by mixing ambient air with compressed CO_2 gas using SmartTrak 50 (Sierra Instruments Ltd.) mass flow controllers coupled with a mixing tee (Figure 2.2). The mixing tee allows homogenous mixing of CO_2 injected into the air before supplied to CO_2 sensors and microcosms. The mixed gas output was monitored and recorded with gas sensors COZIR (Gas Sensing Solutions Ltd.) or LI-80 CO_2 sensor (LICOR). Both sensors were routinely calibrated with premixed compressed 750 ppm CO_2 gas (CalGaz Ltd.). CO_2 readings were recorded per second using the software provided by respective CO_2 sensor manufacturers, and daily averages for this were calculated using R software.

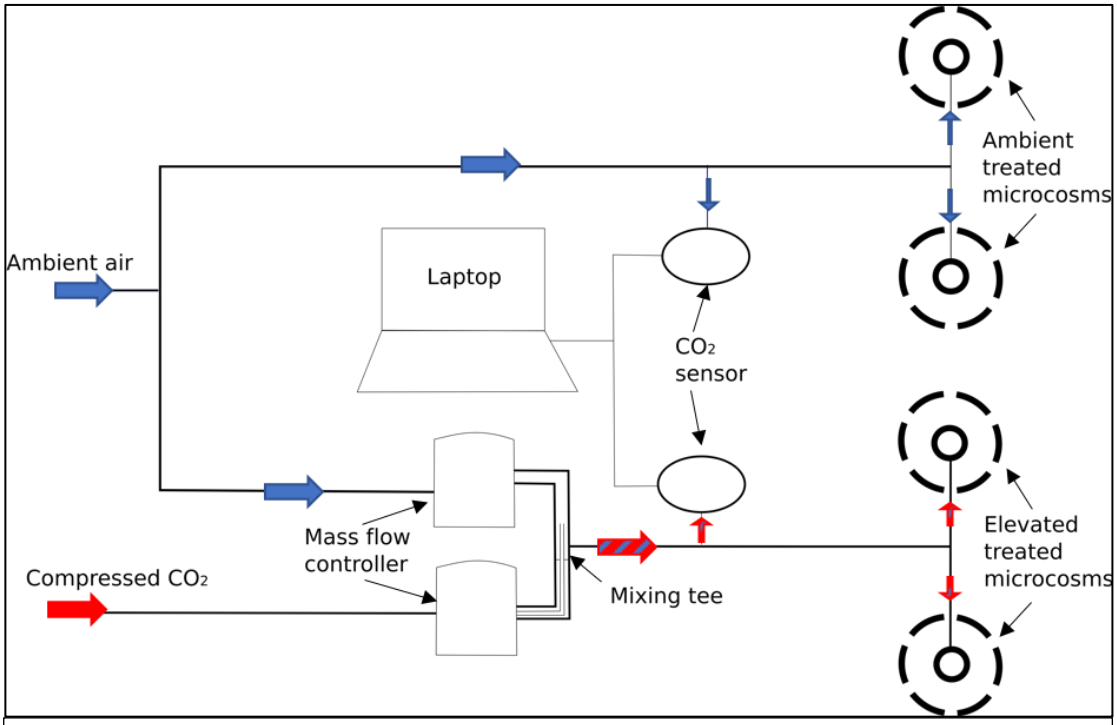


Figure 2.2. Schematic drawing of microcosm aeration for ambient and ocean acidification treatment. Ambient air flow indicated by blue arrows, while elevated CO_2 supplied from compressed gas cylinder indicated by red arrows, while red arrows with blue dash shows mixed air.

The inlets of each mass flow controller were fitted with a 0.22 µm filter to prevent bacterial contamination, dust, and moisture from damaging the equipment. Both ambient and mixed air were supplied to the microcosms via six-way splitters that allows equal distribution of air, and easy manipulation of flow rates into each microcosm. Air flow was monitored visually and adjusted to ensure equal bubbling rates between each microcosm.

2.2.4. Crude oil enrichment

For crude oil enrichments, Schiehallion crude oil which was provided by BP from the Schiehallion oil field in the FSC that was in operation between 1998 to 2013 prior to suspension and refurbishment of the Schiehallion FPSO vessel. Crude oil of 1% (v/v final concentration) was directly added to microcosms during the exponential growth phase of acclimated cultures of *E. huxleyi*, while the same crude oil of the same concentration was added to natural community experiments after 4 days of acclimation to ambient and elevated CO_2 treatments. 1% (v/v) was used to mimic a thin layer of oil typical of an oil spill. Furthermore, 1% (v/v) of crude oil concentration used for optimizing biodegradation of crude oil by marine bacteria (Das and Chandran, 2011; Mishamandani *et al.*, 2015; Farag, Soliman and Abdel-Fattah, 2018).

2.3. Non-axenic cultures of microplankton

All glassware and the inlet caps were rinsed with the dichloromethane (DCM), cleaned with soap and water, and acid-washed by soaking in 1% hydrochloric (HCl) solution overnight before rinsing with deionized water at least 3 times. DCM is a polar solvent that removes any oil residues on the glass surface inside the glassware. Detergent and water ensures thorough cleaning and removes any leftover DCM in the glass bottle and caps, while 1% HCl overnight soak removes any residues of metals, salts, and organic compounds, such as the calcium carbonate shells deposited by coccolithophores that may be bound to the surface of the glass (Dong and Kim, 2018). Clean and dried culturing vessel parts were assembled then sterilized by autoclaving at 121 ^{0}C for 15 minutes. Seawater media and inoculum were added into the room-temperature sterilized culturing vessels.

Agitation to encourage vertical mixing was provided by bubbling of ambient or elevated CO_2 directly into each microcosm. These microcosms consist of batch cultures of non-axenic microalga in an open vessel with headspace in order for seawater carbonate

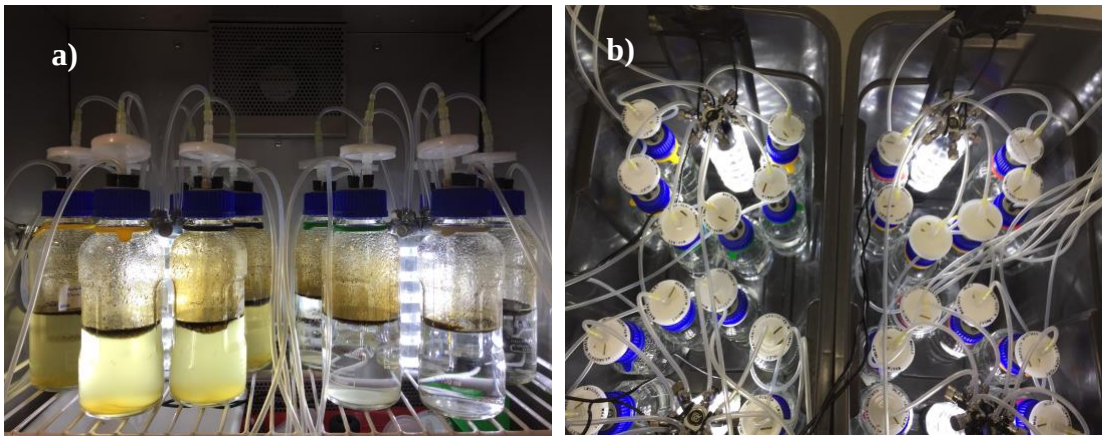


Figure 2.3. Microcosm arranged inside temperature-regulated containers. **(a)** Laboratory incubator. **(b)** Water baths during on-board culturing. Water in each waterbath was pumped out to a portable chiller using an aquarium pump.

chemistry to be in equilibrium with CO_2 in the air at standard atmospheric pressure for stable manipulation of carbonate chemistry (Riebesell *et al.*, 2011). Culturing media in the microcosm were not constantly stirred in order to avoid damage to cells from turbulence (Riebesell *et al.*, 2011). In order to maintain a stable temperature, this was regulated using temperature-controlled dry-air incubators, or water baths (Figure 2.3). An aquarium pump was used to constantly pump water from the water bath to the chiller. The chillers were set according to the temperature of the seawater during sampling.

2.3.1. E. huxleyi monoculture

A non-axenic culture of *E. huxleyi* strain 920/8 was used in this experiment. The strain was obtained from the Culture Collection for Algae and Protozoa (CCAP, Oban, UK). The strain had been isolated from a Bergen mesocosm in Norway and reported to host oil-degrading bacteria, such as *Alcanivorax* and *Marinobacter* (Green *et al.*, 2015). Strain 920/8 was maintained in filtered natural seawater that was pre-autoclaved and enriched with f/2 nutrient amendment (Guillard and Ryther, 1962) with additional supplementation with selenium (Danbara and Shiraiwa, 1999). The f/2 medium was prepared in 10-L carboys that were autoclaved, allowed to cool and then mixed before aliquoting the medium into each sterile microcosm. For this, 300-ml aliquots of the medium were transferred under a laminar flow hood into each microcosm to prevent microbial contamination.

Stock cultures were acclimated to ambient air or elevated CO_2 conditions and maintained for several months prior to starting the experiments. Cultures were maintained at 14 ^{0}C using incubators (Panasonic). Prior to experiment, a sample of exponentially growing ambient-acclimated and elevated-CO_2 acclimated cultures were counted using a haemocytometer chamber to determine the cell concentration. This allowed for the same starting concentration of algal cells from the CO_2 acclimated stocks into their respective microcosms to begin the experiments. Acclimation to treatment and subculturing of the pre-treated algal stock cultures into experimental microcosms reduces lag phase of algal growth (Fogg and Thake, 1987).

2.3.2. Natural community of microplankton

Natural community of plankton were sampled from 5m below the surface seawater of the Faroe-Shetland Channel (FSC) using Niskin bottles arranged on a rosette sampler coupled with a SeaBird CTD and filtered using a 120 μm mesh to remove larger plankton and grazers. The FSC is a subarctic region of the northeast Atlantic located to the west of the North Sea. It is a site of interest to the oil and gas industry. BP announced to double the production of hydrocarbon from its Schiehallion oil field and the adjacent Loyal oil field by 2020 (BP Plc, 2017).

Thus, the new Glen Lyon floating, production, storage, and offload (FPSO) vessel was refurbished and expanded and began operations after a 4-year hiatus to increase oil recovery from Schiehallion and Loyal oil field. It is recognized as the world's largest harsh water FPSO and is expected to produce oil at a rate of 130,000 barrels per day, up to the year 2035 (BP Plc, 2017). Crude oil from the Schiehallion oil field was used for oil enrichment in all of the experiments carried out in this thesis.

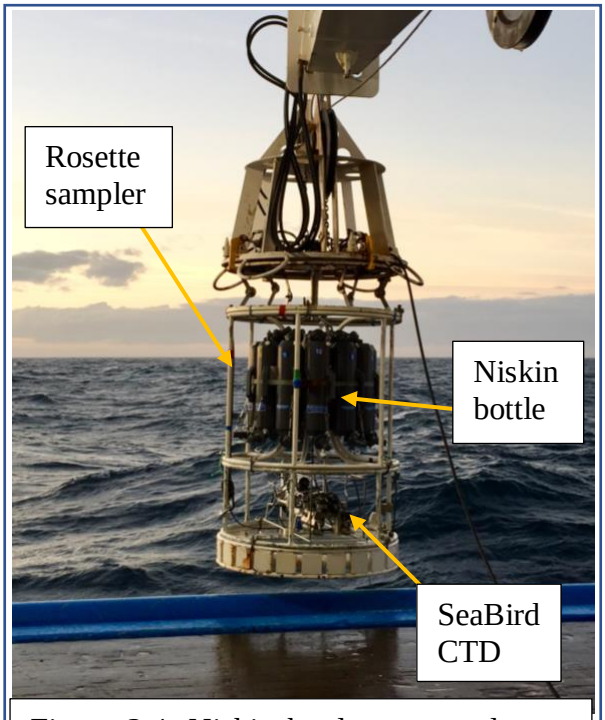


Figure 2.4. Niskin bottles arranged on a rosette sampler for seawater sampling. SeaBird CTD records temperature, depth and salinity during sampling.

With major hydrocarbon fields and pipelines in the area expected to be running at peak capacity in the next couple of decades, including the recent expansion of Clair field for second-phase of development, the FSC is an important region for investigating the response, resilience and function of oil-degrading microbial communities in the event of a potential major oil spill in this region. Oil production resumes in 2018, and peak production is expected to be 100,000 barrels per day (BP Plc, 2017).

Regular oceanographic cruises to this area since 1903, with the exception of a couple of years due to the break out of war, had provided a substantial baseline for physicochemical conditions such as salinity, temperature, primary production, nutrient parameters, and established flow of the water masses that travels through the site that drives the Meridional Overturning Circulation; great ocean conveyer belt (Berx, 2012). In recent years, bacterial community baseline in the Faroe-Shetland have been described

with particular interest in hydrocarbon-degrading bacteria and response to dispersant (Suja, Summers and Gutierrez, 2017; Perez Calderon *et al.*, 2018).

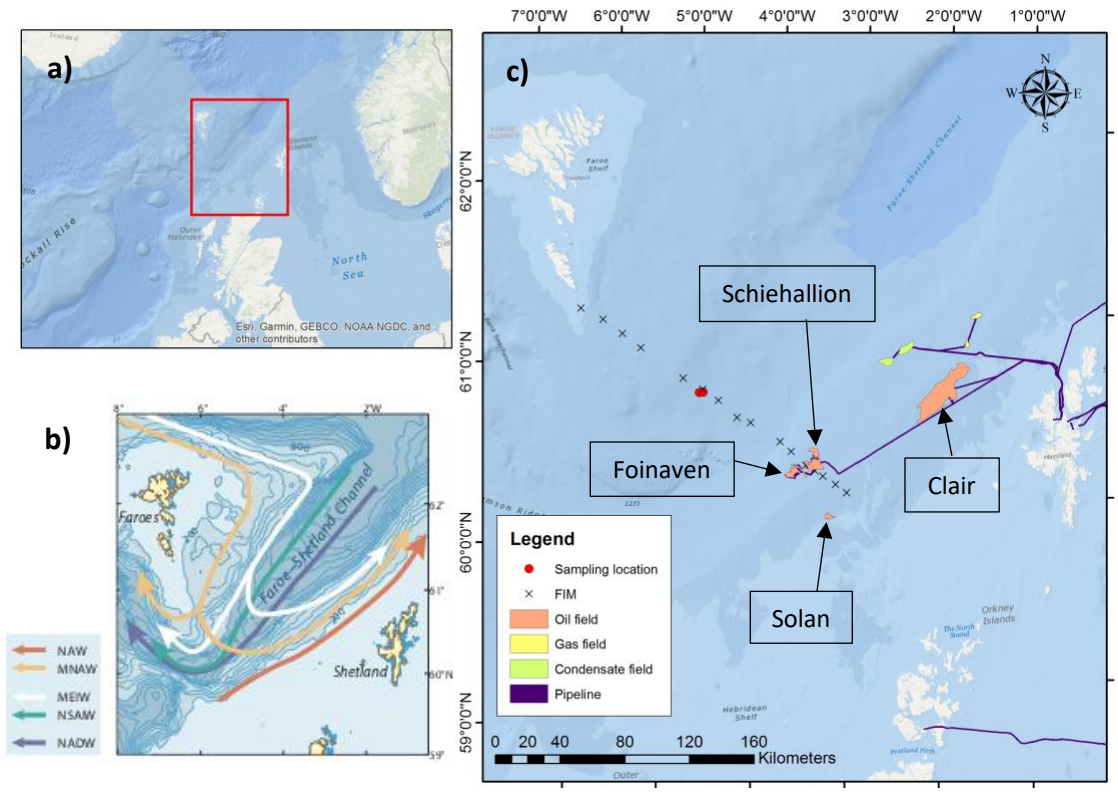


Figure 2.5. Sampling location of natural community of phytoplankton in FSC. **(a)** Location of Faroe-Shetland Channel indicated by area of red square enlarged in (c). **(b)** Schematic representation of water masses flowing through FSC, NAW; North Atlantic Water, MNAW; Modified North Atlantic Water; MEIW; Modified East Icelandic Water, NSAIW; Norwegian Sea Intermediate Water, NADW; North Atlantic Deep Water (adapted from Berx, 2012). **(c)** Sampling location relative to FIM transect oceanography survey (Marine Scotland, 2017a), hydrocarbon fields (*OGA Offshore FieldDets WGS84*, 2018) and pipelines (Marine Scotland, 2017b). Map (a) and (c) presented in WGS84 / UTM zone 29N projected coordinate system on basemap of Worlds Ocean (Esri).

Seawater samples were collected in the spring of May 2017, and during the fall of October 2017 during a survey along the Fair Isle-Munken (FIM) transect line carried out by Marine Scotland Science. Figure 2.5 shows the sampling location in the FSC, including the oil fields and a schematic representation showing the five distinct water masses that define the water column at this site which are generally characterised by temperature-salinity properties. The water above 400 m depth is a mixture of North Atlantic Water (NAW) and Modified North Atlantic Water (MNAW), which are the warmest and saltiest of the five water masses (Berx, 2012). Samples of the first 5 meters of the MNAW water mass was taken for both spring and fall experiments. These samples were filtered to remove grazers using a 120 μm mesh and the filtrate placed into a clean and sterile 10 L carboy for homogenous mixing, before 400 ml was aliquoted into pre-

autoclaved 500 ml Duran Youtility glass bottles (Figure 2.1). Triplicates of 50 ml of seawater from the site was preserved with Lugol's iodine for taxonomic analysis and cell counts of phytoplankton cells. This was performed at the Scottish Environment Protection Agency (SEPA) for samples collected during the May 2017 cruise, and by Marine Scotland Science for samples collected during the October 2017 cruise.

During on-board experiments, water baths connected to chillers were used to maintain the surface-seawater temperature of 10 ^{0}C for spring experiment and 11 ^{0}C for fall experiment. The surface seawater temperature was measured using the SeaBird CTD attached to the rosette sampler during sampling. Light conditions and agitation by bubbling of air or elevated CO_2 were as mentioned previously in section 2.2.2. Cultures were acclimated to CO_2 treatments for 4 days prior to amendment with crude oil. The microcosms were maintained onboard MRV *Scotia* until the end of the cruise: day 9 for spring experiment, and day 8 for fall experiment. Samples were then transferred to laboratory on the same day while being transported in an airtight container. Aeration was discontinued for at least 5 hours during transit between the port to the laboratory and was immediately aerated as soon as gas mixing setup was reassembled in the laboratory.

2.4. Sampling and Analysis

2.4.1. Chlorophyll a concentration

Chlorophyll *a* (chl *a*) concentrations were determined as a proxy for the abundance of *E. huxleyi* cells and of phytoplankton from field samples. Chl *a* was extracted using a modified version of EPA 445 (Arar and Collins, 1997) by pelleting 2 ml of homogenized sample. To prevent contamination from hydrocarbons, samples taken from microcosms were pelleted by centrifugation and the resultant supernatant removed to leave behind hydrocarbons/crude oil that adhered to the plastic centrifugation tubes and tips. The cell pellets were carefully transferred to a clean tube and resuspended in 1ml of 90% acetone and stored in the dark at -20 ^{0}C overnight. Samples were centrifuged to collect the supernatant for fluorometric analysis using a Turner Trilogy Fluorometer (Turner Designs, CA 94085) with excitation wavelength at 485 nm and emission at 685 nm. The primary chl *a* calibration standard was configured using known concentrations of spinach extract as recommended by Welschmeyer, (1994) and stored in the fluorometer to be routinely checked with solid state secondary standard (Turner Designs, CA 94085) prior to analysis.

2.4.2. Bacterial community analysis

Baseline community (t0) was sampled directly from the sampling location by filtering 5 ml of seawater collected from the FSC on 45 mm polycarbonate membrane filters (0.22 μm; Isopore) and stored at -20 ^{0}C for subsequent extraction of DNA. Comparison of baseline samples with microcosm samples allows identification of taxa affected by bottling effect (Ferguson, Buckley and Palumbo, 1984). Samples from microcosms were then extracted at three timepoints: 1) acclimation to CO_2 treatments/4 days exposure to CO_2 treatments (t1); 2) at least a week after oil enrichment (t2); and 3) at the end of experiment/2-weeks after oil enrichment (t3). Exact timepoints of t2 and t3 DNA sampling for each experiment were mentioned in section 4.2.4 (spring community) and in section 5.2.4 (fall community). Similar to baseline community, 5 ml of samples from microcosms at each timepoint were extracted and stored at -20 ^{0}C.

The filters were cut into three equal parts and placed into 1.5 ml microcentrifuge tubes and ground using liquid nitrogen and crushing filters using a sterile pipette tips. Liquid nitrogen was allowed to evaporate completely before contents were extracted using the potassium ethyl xanthogenate protocol (Tillett and Neilan, 2000). Extracted DNA were stored at -20 ^{0}C before amplification of 16S rRNA.

Targeted sequencing of the conserved 16S ribosomal RNA region allows identification of bacterial community composition from large number of samples with the greatest coverage at a relatively low-cost (Caporaso *et al.*, 2012a). Targeted sequencing of the V4 region using 515f (5'-GTGYCAGCMGCCGCGGTAA-3') and a complementary 806RB reverse primer (5'-GGACTACNVGGGTWTCTAAT-3') primers improves detection of clade SAR11 in marine samples without affecting detection of taxa previously identified in region 515f/926r.

The V4 region of 16S rRNA gene was amplified using Golay barcoded 515F and 806R primers according to the 16S Illumina amplicon protocol recommended by Earth Microbiome Project (Apprill *et al.*, 2015; Caporaso *et al.*, 2018). Amplification was done in triplicates of 75 μl reactions. PCR was carried out using Platinum Hot Start PCR Mastermix (ThermoFisher Scientific). Thermocycler conditions were 94 ^{0}C for 3 minutes, 35 cycles of 94 ^{0}C for 30 seconds, 50 ^{0}C for 60 seconds, 70 ^{0}C for 90 seconds, and a final extension step at 72 ^{0}C for 10 minutes. PCR products were purified using GE Healthcare PCR purification kit. Quantification of DNA concentration and A260/A280 ratio from each sample were done using NanoDrop. Only samples that gave A260/A280 ratio of 1.8 – 2.0 were added into the pool in equal amounts (Caporaso *et al.*, 2012b).

Samples were pooled and sent for Illumina MiSeq sequencing at the Edinburgh Genomics Facility.

Libraries of bacterial 16S rRNA gene sequences for each barcoded sample were constructed for OTU-based analysis using mothur version 1.39.5 (Schloss *et al.*, 2009). Contiguous sequences from paired end sample reads were constructed, and homopolymers more than 8 bases long were removed (Kozich *et al.*, 2013). Chimeras were removed and cleaned sequences were classified and referenced to the RDP database (version 16), and similarities below 80% were removed. The output of the classified OTU's from mothur were analyzed by pairwise comparison of heat tree plots using metacodeR (Foster, Sharpton and Grünwald, 2017). OTU reads that were less than 5 base-pairs were removed before data was transformed to proportions to avoid sampling depth bias. Each taxon abundance was calculated and a Wilcox rank-sum test and a Benjamini Hochberg (FDR) correction for multiple comparisons were used to determine for significant differences in taxa between the various treatments (Foster, Sharpton and Grünwald, 2017).

2.4.3. Crude oil extraction

Microcosms used for hydrocarbon analysis were not sampled at any time until at the termination of these experiments when they were sacrificed for extraction using HPLC-grade dichloromethane (DCM). Liquid phase separation was carried out to separate crude oil from the seawater, and the DCM evaporated using a rotary evaporator in a water bath at 40 ^{0}C. The extracted oil was stored in amber glass vials with Teflon screw caps at -20 ^{0}C prior to hydrocarbon analysis.

GC-FID was used to analyze for aliphatic species in the crude oil. Ratios of aliphatic compounds, *n*C17/pristine and *n*C18/phytane were analyzed for biological degradation compared to degradation caused by abiotic factors (Olson *et al.*, 2017). Similarly, aromatic ratios for biodegradation were compared for naphthalene/2-methylnaphthalene (N/2-MN), 2-methylnaphthalene/1-methylnaphthalene (2MN/1-MN), 2-ethylnaphthalene/2,6+2,7-dimethylnaphthalene (2-ET/(2,6+2,7)-DMN), 2-methylnaphthalene/2,6+2,7-dimethylnaphthalene, phenanthrene/9-methylphenanthrene (P/9-MP), 3+2-methylphenanthrene/9+1-methylphenanthrene (3-MP + 2-MP/9-MP + 1-MP), and 3-methylphenanthrene/9-methylphenanthrene (3-MP/9MP). One-way ANOVA was performed on these ratios to determine significant differences of hydrocarbon degradation between treatments. Atmospheric pressure photoionisation (APPI) coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) were

carried out and grouped into classes based on their heteroatom content (Lemkau *et al.*, 2014; McKenna *et al.*, 2013). Mass spectral peaks were normalized to their most abundant peaks in each mass spectrum and changes across heteroatom classes visualized by isoabundance plot of double bond equivalence (number of rings plus double bonds to carbon) against carbon number were plotted (Lemkau *et al.*, 2014). Therefore changes seen in each heteroatom class is the result of both degradation and formation of new stable compounds (Lemkau *et al.*, 2014).

2.4.4. Analysis of carbonate chemistry

Total dissolved inorganic carbon (DIC), total hydrogen ion (pH_t), temperature, and salinity were measured for each microcosm experiment, as described below. By combining these parameters, other variables of the carbonate system such as total alkalinity, bicarbonate ion concentration, carbonate ion concentration, calcite saturation, and aragonite saturation could be calculated (Dickson, Sabine and Christian, 2007). Seawater collected from the FSC in Niskin bottles, and seawater or growth medium (f/2) from microcosms sampled at the end of experiments were preserved in mercuric chloride and stored in the dark at room temperature with minimal head space. Narrow-necked borosilicate glass bottles (100 ml) were filled to the top with media from microcosm leaving 1% headspace as described in SOP1 to allow for expansion of water (Dickson, Sabine and Christian, 2007). Samples were preserved by adding saturated mercuric chloride to samples for a final concentration of 0.02% to prevent biological activity that might affect carbonate chemistry, and sealed with a silicon-free grease (Apiezon) lined on the sides of the glass stopper to prevent gas exchange between samples and air in the atmosphere of the storage container. These samples were kept at 4 ^{0}C in the dark until ready for analysis. Prior to analysis, bottles containing samples were incubated in a water bath to ensure constant temperature between samples and standard (Dickson, 2011).

Total DIC was determined by acidification, gas stripping, and infrared detection using a DIC analyzer Model AS-C3 (Apollo SciTech Inc.) coupled with Li-7000 CO_2/H_2O Analyzer (Li-COR). DIC instruments were calibrated using seawater reference material batch 142 provided by Scripps Oceanography (Dickson, 2014). 10% of phosphoric acid in 0.1% (w/v) of sodium chloride in deionized water were used to acidify samples by titration for the release of CO_2 and for the determination of total DIC.

Total hydrogen ion of samples was determined by electrometric determination using the Orion ROSS Ultra pH/ATC (ThermoFisher) glass electrode with a standard 2-amino-2-methyl-1,3-propanediol (Tris) buffer in synthetic seawater. The electromotive

force (e.m.f) measurements of sample determined the pH according to the equation below,

$$pH(X) = pH\ (S) - \frac{E_x - E_s}{RTln10/F}$$

where pH(X) is the pH of sample, pH(S) corresponds to pH of Tris standard, E_x and E_s are e.m.f. of sample and standard respectively, R is the gas constant, F is the Faraday constant, and T is the temperature (Dickson, 2011).

Salinity, temperature, total DIC, and total hydrogen ion values of each sample were used to calculate carbonate parameters using co2calc version 1.2 developed by United States Geological Society (Robbins, Hansen and Meylan, 2016). Two-way ANOVA and multiple comparisons test was done to compare carbonate parameters between treatments.

Chapter 3: Impacts of ocean acidification and crude oil pollution on *Emiliania huxleyi's* bacterial consortia

3.1. Introduction

Emiliania huxleyi is a coccolithophore that have been found widely distributed globally with the exception of polar seas and is a major source of particulate inorganic carbon (PIC) in the surface waters in large scales detectable by satellite imaging (Holligan, Charalampopoulou and Hutson, 2010). Field observations of coccolithophores shows they have benefited from OA over the last decade from the increasing levels of dissolved CO_2, resulting in an increase in chl *a* concentration in the subtropical region, even if calcification is hindered by OA (Krumhardt *et al.*, 2016). Laboratory studies of the coccolithophore *E. huxleyi* reported inconsistencies in response to OA due to strain specific responses as a result of pan-genomic variability (Iglesias-Rodriguez *et al.*, 2008; Riebesell *et al.*, 2008; Read *et al.*, 2013). The variable region of *E. huxleyi* genome includes genes affecting calcification and photosynthesis, such as phosphate transporters, alkaline phosphatase, and iron-binding proteins (Read *et al.*, 2013). This pan-genomic variability of *E. huxleyi* likely accounts for their global distribution and abundance during phytoplankton blooms.

Coccolithophores were reported to host a bacterial community relatively more diverse than dinoflagellates and diatoms due to the complex biochemical and biophysical environment from the actively growing and calcifying coccolithophores as opposed to dinoflagellates (Green *et al.*, 2015). *Phaeobacter inhibens*, a bacteria associated to *E. huxleyi*, were found to play a major role in the microalgae's abundance through symbiotic and parasitic interactions (Seyedsayamdost *et al.*, 2011; Segev *et al.*, 2016; Bramucci *et al.*, 2018). *P. inhibens* produces roseobacticide that promotes alkenone lipid production in *E. huxleyi* during senescence, but changes its role from an antagonist to a protagonist by promoting algal growth depending on physicochemical properties of the seawater such as nutrient availability, N:P ratio, and light intensity (Seyedsayamdost *et al.*, 2011; Fuentes *et al.*, 2016; Segev *et al.*, 2016).

Other than alkenone lipids, *E. huxleyi* produces long-chained alkenes, long chained alkenones, phospholipids, and glycolipids (Yamane *et al.*, 2013). Microbiota of marine phytoplankton have been identified capable of degrading hydrocarbon (Gutierrez *et al.*, 2012; Gutierrez, Green, *et al.*, 2013; Green *et al.*, 2015). Since *E. huxleyi* thrives in oligotrophic conditions, they could be seen as seeding nutrients or harbouring bacterial communities in low nutrient conditions that have the potential to respond to crude oil pollution.

Bulk of crude oil are n-alkanes are primarily removed by bacteria. A significantly higher percentage of hydrocarbon and toxic PAH are introduced into the ocean from

maritime activities and fossil fuel excavation. Although oil is a carbon substrate, it lacks other microbially important nutrients such as nitrogen and phosphorus making nutrient limitation of hydrocarbon degrading communities a determining factor in oil degradation. This study investigates the response of cosmopolitan *E. huxleyi* and its associated bacterial community to hydrocarbon pollution under future ocean conditions (750 ppm CO_2) to address the impact of ocean acidification on microbial communities with the potential of degrading crude oil.

3.2. Methods

3.2.1. Culturing conditions

A monospecific culture of non-axenic *Emiliania huxleyi* strain 920/8 obtained from CCAP were cultured in filtered natural seawater, autoclaved and enriched with f/2 nutrient amendment (Guillard and Ryther, 1962). The *E. huxleyi* strain 920/8 used in this experiment was provided by the CCAP, isolated from a Bergen mesocosm in Norway and have been characterised as a host to oil degrading bacteria, *Alcanivorax* and *Marinobacter* (Green *et al.,* 2015). This experiment utilises microcosm batch cultures as described in Chapter 2, to manipulate carbonate chemistry of seawater media in microcosm and to allow total hydrocarbon extraction at the end of experiment. Cultures were pre-conditioned at 14 ^{0}C, ambient light of 50 μmol m^{-2} s^{-1} at 16:8 h light:dark cycles, at two separate atmospheric CO_2 conditions by bubbling ambient; 400 ppm CO_2 and elevated; 750 ppm CO_2 directly into the stock cultures.

E. huxleyi were acclimatised in 750 ppm CO_2 for ~100 generations prior to experiment. Acclimatised *E. huxleyi* were then diluted into microcosms subjected to their respective CO_2 conditions. After 5 days of initial inoculation with *E. huxleyi* (during exponential growth phase), microcosms subjected to crude oil enrichment were enriched with 1% v/v Schehallion crude oil. Each atmospheric CO_2 condition went through 4 treatments, carried out in triplicates. Treatments include 1) a non-inoculated control with crude oil enrichment, 2) crude oil enrichment on exponentially growing *E. huxleyi* for biological sampling, 3) crude oil enrichment on exponentially growing *E. huxleyi* (hydrocarbon analysis), and 4) acid-killed control; phosphoric acid added (pH= 1) to kill *E. huxleyi* at exponential phase prior to oil enrichment (hydrocarbon analysis).

Due to the limitation in space for culturing *E. huxleyi* with ambient and elevated CO_2 in microcosms, a supplementary experiment was carried out to investigate growth rate of *E. huxleyi* without oil enrichment, and oil degradation without biological influence. Supplementary experiment was carried out using the same seawater,

autoclaved on the same day of the previous experiment and stored at 4 ^{0}C in the dark, a supplementary experiment with same CO_2 conditions was carried out for 1) biological control (*E. huxleyi* without oil) 2) non-inoculated control with crude oil enrichment (hydrocarbon analysis), 3) acid-killed control prior to oil enrichment, and 4) non-inoculated without oil (f/2 amended seawater).

Microcosms subjected to hydrocarbon analysis in both the main experiment as well as the supplementary experiment were not sampled/left untouched until the end of experiment to in order to attain total hydrocarbon from microcosm and to prevent accidental removal of crude oil during sampling.

3.2.2. Growth rate of E. huxleyi

Enumeration of *E. huxleyi* was done by cell count using a haemocytometer viewed under a light microscope prior to oil enrichment, in addition to extraction of chlorophyll *a* (chl *a*) fluorometric analysis taken from day 0 to day 4. Growth rates (μ) were determined by plotting ln of cell counts from day 0 to day 4 of nine replicates and acquiring the gradient of the line of best fit (Stanbury, Whitaker and Hall, 1995). The rationale of using the gradient of line of best fit between abundance determined on day 0 and abundance on day 4 can be explained by the equation below (Stanbury, Whitaker and Hall, 1995),

$$dN/dt = \mu N$$

where N is the number of cells, t is time, and d is the change/difference of cells (N) or time (t). The proportion of change of number of cells over change of time was expressed as μ or growth rate of population of N.

$$\mu = \ln A_f - \ln A_i / t_f - t_i$$

where A is the abundance determined by cell concentration per mL, t is time of the exponential phase measured up to day 5 prior to oil enrichment. The subscripts i and f are initial and final timepoints, respectively of cell concentration.

After oil enrichment, only chl *a* extraction was carried out due to efficiency in separating crude oil from samples by pelleting cells using a centrifuge. Chl *a* were extracted by pelleting 2 ml of homogenised sample and transferred to a clean tube containing 1ml of 90% acetone stored in the dark at -20 ^{0}C overnight. Samples were then centrifuged, and the supernatant was transferred into clean glass vials for fluorometric analysis at an excitation wavelength of 485 nm and emission at 685 nm using Turner Trilogy Fluorometer (Turner Designs, CA 94085).

3.2.3. Bacterial community analysis

Microcosms were lightly shaken and 5 ml of homogenised sample were extracted at three timepoints, t0: prior to oil enrichment (day 5), t1 + oil; 1 day after oil enrichment (day 6), and t2+oil; 17 days after oil enrichment (day 21). In addition, samples from supplementary experiment were taken on day 8 (t1 – oil) and day 15 (t2 - oil) of culturing without oil. The samples were filtered on a 45mm polycarbonate membrane filters of 0.22 μm pore and stored at -20 ^{0}C. DNA extraction was carried out using potassium ethyl xanthogenate method described by Tillett and Neilan (2000) as mentioned in Chapter 2. PCR amplification of the V4 region of 16S rRNA were carried out using Platinum Hot Start PCR Mastermix (ThermoFisher Scientific). Thermocycler conditions were 94 ^{0}C for 3 minutes, 35 cycles of 94 ^{0}C for 30 seconds, 50 ^{0}C for 60 seconds, and 70 ^{0}C for 90 seconds, and a final extension step at 72 ^{0}C for 10 minutes. PCR products were cleaned using GE Healthcare PCR purification kit before samples were pooled and sent for Illumina MiSeq sequencing by Edinburgh Genomics.

Post-sequencing analysis was carried out using mothur version 1.39.5 (Schloss *et al.*, 2009) where contiguous sequences were constructed, and homopolymers more than 8 bases long were removed as detailed in Chapter 2. The sequences aligned against SILVA database, chimeras were removed and sequences that was left were classified and referenced to RDP database (version 16). Only sequences > 80% similar were used. Alpha-diversity was calculated using Shannon diversity index (H') while beta-diversity was assessed using Bray-Curtis similarity index visualised on an NMDS plot. Pairwise comparison of each taxa in respected treatments were analysed using ratios of log2 median proporotions of taxa between treatment and visualised using heat-tree plots made using metacodeR R package (Foster, Sharpton and Grünwald, 2017).

3.2.4. Hydrocarbon extraction and analysis

Crude oil from untouched microcosms were extracted using HPLC grade dichloromethane (DCM) followed by liquid phase separation and evaporated using rotary evaporator in a water bath at 40 ^{0}C. The extracted oil was stored in amber glass vials with Teflon screw caps at -20 ^{0}C prior to gas chromatography-flame ionisation detector carried out by Bernard Bowler from University of Newcastle. Ratios of aliphatic compounds, nC17/pristine and nC18/phytane were analysed for differentiating biological degradation from weathering (Olson *et al.*, 2017). Similarly, aromatic ratios for biodegradation was compared for naphthalene/2-methylnaphthalene (N/2-MN), 2-methylnaphthalene/1-methylnaphthalene (2MN/1-MN), 2-ethylnaphthalene/2,6+2,7-dimethylnaphthalene (2-

ET/(2,6+2,7)-DMN), 2-methylnaphthalene/2,6+2,7-dimethylnaphthalene, phenanthrene/9-methylphenanthrene (P/9-MP), 3+2-methylphenanthrene/9+1-methylphenanthrene (3-MP + 2-MP/9-MP + 1-MP), and 3-methylphenanthrene/9-methylphenanthrene (3-MP/9MP). One-way ANOVA was carried out on ratios to determine significant differences of hydrocarbon degradation between treatments.

3.2.5. Carbonate chemistry analysis

Samples were preserved in 0.02% mercuric chloride and kept in glass bottles with a narrow neck, sealed with a glass stopper and silicone free grease (Apiezon) according to SOP 1 of Guide to Best Practices for Ocean CO_2 Measurements (Dickson, Sabine and Christian, 2007). These samples were stored at 4 ^{0}C in the dark prior to analysis. Total dissolved inorganic carbon and total hydrogen ions were analysed as previously described in Chapter 2. Two-way ANOVA and Tukey's multiple comparison test were used to determine significance of carbonate parameters such as total CO_2, total pH, bicarbonate ion concentration, carbonate ion concentration, aragonite saturation and calcite saturation.

3.3. Results

3.3.1. E. huxleyi's response to ocean acidification and oil pollution

Growth rate of *E. huxleyi* from slope of (Figure 3.1) shows no significant differences ($p= 0.57$) between elevated CO_2 (0.99 day^{-1}) and ambient CO_2 (1.06 day^{-1}; Figure 3.1). Prior to oil enrichment, no significant differences of chl *a* were seen between ambient treated microcosms and elevated CO_2 treated microcosms on day 2 ($p = 0.994$) and day 3 ($p = 0.9644$) when tested with 2-Way ANOVA and multiple comparison test (Figure 3.2). Furthermore, no significant differences between chl *a* concentration in ambient treated *E. huxleyi* prior to oil enrichment, compared to ambient treated *E. huxleyi* prior to acid killing (day 2; $p > 0.999$, day 3; 0.9986). This was also seen in elevated treated *E. huxleyi* prior to oil enrichment, compared to *E. huxelyi* prior to acid-killing (day 2; $p > 0.999$, day 3; $p = 0.9973$).

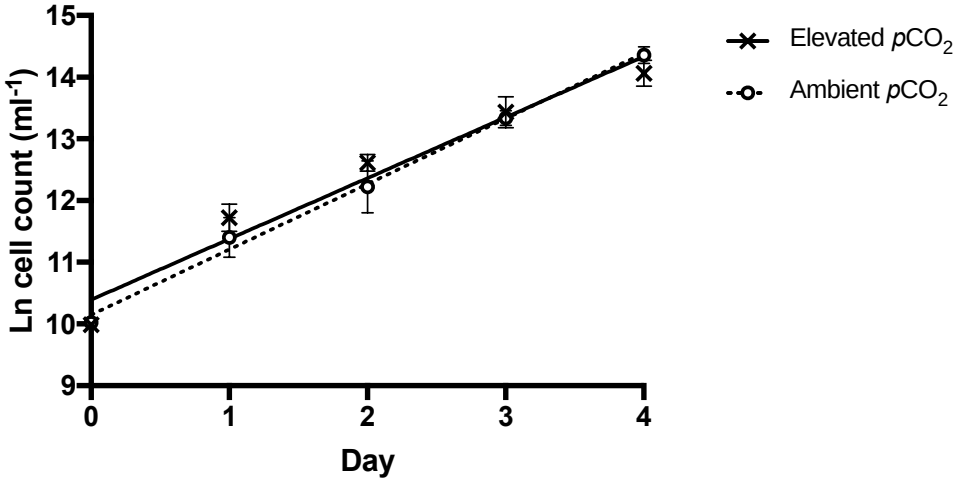


Figure 3.1. Growth rate of *E. huxleyi* exposed to elevated and ambient pCO_2 conditions prior to oil enrichment shown by the slope of natural log of cells per ml over time (days). Slope of cell counts grown under elevated pCO_2 (0.99 ± 0.12) were identical to slope of cell counts grown ambient pCO_2 (1.06 ± 0.04).

On day 4, microcosms that were treated with ambient CO_2 showed significantly lower chl *a* concentration compared to elevated CO_2 treated microcosms ($p = 0.0002$). Chl *a* concentration showed cultures grown under elevated conditions doubled on day 5, prior to oil enrichment and is significantly higher than ambient treated microcosms ($p < 0.0001$; Figure 3.2). Chl *a* concentration in microcosms treated with ambient CO_2 prior to oil enrichment was not significantly different from ambient treated CO_2 prior to acid killed control on day 5 ($p = 0.9987$). Similarly, chl *a* concentration in microcosms treated with elevated CO_2 prior to acid or crude oil exposure were not significantly different ($p = 0.9971$).

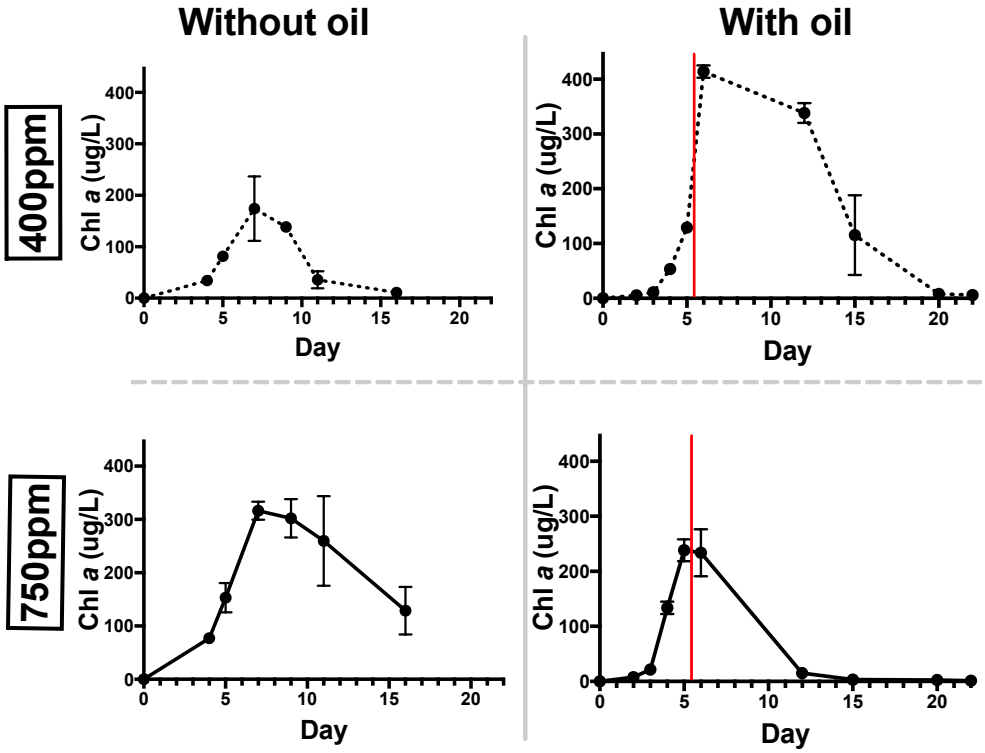


Figure 3.2. Growth response of *E. huxleyi* in microcosm cultures treated with ambient and elevated (750 ppm) CO_2. pH levels of microcosms with *E. huxleyi* enriched with crude oil after day 5 (red line) in ambient (top;400ppm) and elevated (bottom;750ppm) CO_2 conditions and uninoculated control enriched with crude oil (open circle). Values are averages of triplicate incubations with error bars showing standard error of measurement (SEM) except for day 7, 11 and 16 of ambient/without oil where values were duplicates.

E. huxleyi in elevated CO_2 treated microcosms immediately entered stationary phase after oil enrichment, followed by relatively rapid death phase as chl *a* concentration was not detected 10 days after oil enrichment (day 15). Decline in chl *a* from oil-enriched/ ambient treated *E. huxleyi* were delayed as chl *a* concentration on day 12 ($p < 0.0001$) and day 15 ($p < 0.0001$) were significantly higher compared to oil-enriched/elevated CO_2 treated microcosms (Figure 3.2). No significant differences were detected on day 20 and day 22 as chl *a* in both treatments were no longer detected (Figure 3.2).

3.3.2. Bacterial community response to ocean acidification and crude oil enrichment

During post-sequencing analysis using mothur, some libraries were lost as a result of no sequences detected and removal of sequences due to standard quality control during processing of libraries according to the standard MiSeq protocol clarified in Chapter 2.

Libraries that were not included due to loss of replicates were ambient/oil-enriched at t2 (400 ppm + oil t2), ambient/without oil at t1(400 ppm – oil t1), elevated/without oil at t1 (750 ppm – oil t1), and elevated/without oil at t2 (750 ppm – oil t2). Libraries that correspond to treatments that were successfully sequenced, processed for analysis, and in triplicates were analysed as follow.

Species diversity determined with the Shannon diversity index (H') shows a very close association of number of taxa and species evenness between each treatment (Figure 3.3). Tukey's multiple comparison's test and one-way ANOVA shows the differences between H' of ambient/elevated CO_2 at different timepoints, with/without crude oil enrichment were not significantly different ($p < 0.05$; Figure 3.3). Kruskal-Wallis test determined the median between treatments did not vary significantly ($p = 0.6874$).

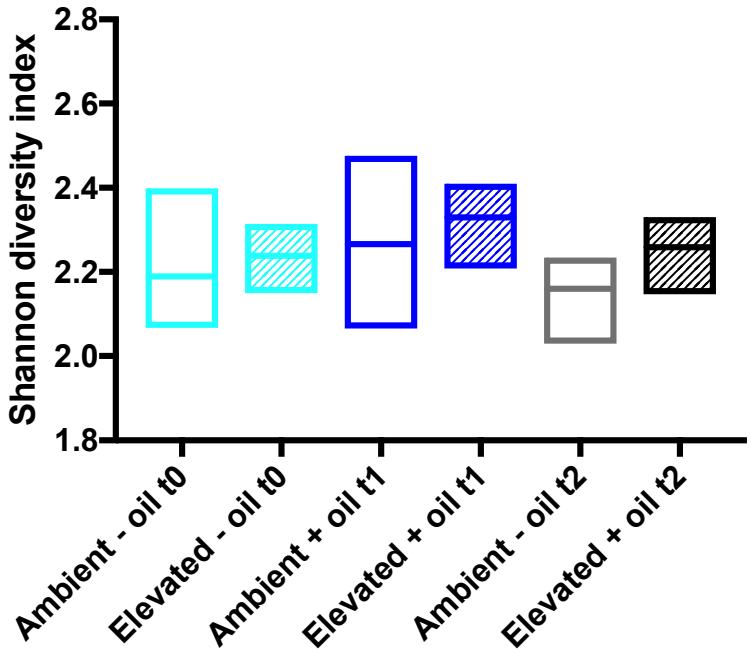


Figure 3.3. Variance of OTU's between treatments were analysed using Shannon diversity indices of shown in floating bar plot. Line shows median value of Shannon diversity, with minimum and maximum values as borders of each bar plot. Clear bars indicate samples treated with ambient conditions at different timepoints, while filled bars shows samples treated with elevated CO_2 at different timepoints.

Comparison of taxa diversity between treatments were carried out using ordination based on the Bray-Curtis similarity matrix of the relative abundance data obtained from MiSeq 16S rRNA gene fragment amplicon sequencing normalised by log(X+1) (Figure 3.4). NMDS ordination concludes sample distribution with respect to two CO_2 treatments (ambient and elevated) in the presence/absence of 1 % crude oil sampled at three timepoints within a period of 22 days were at least 60% similar (Figure

3.5). Samples clustered closely together without any overlaps and dissimilarity of less than 50% indicates bacterial community structure associated with *E. huxleyi* were strongly similar in terms of species composition between treatments. ANOSIM pairwise test identified no statistical differences of community composition (Bray-Curtis) between CO_2 treatments, thus supported the trend identified by NMDS plot.

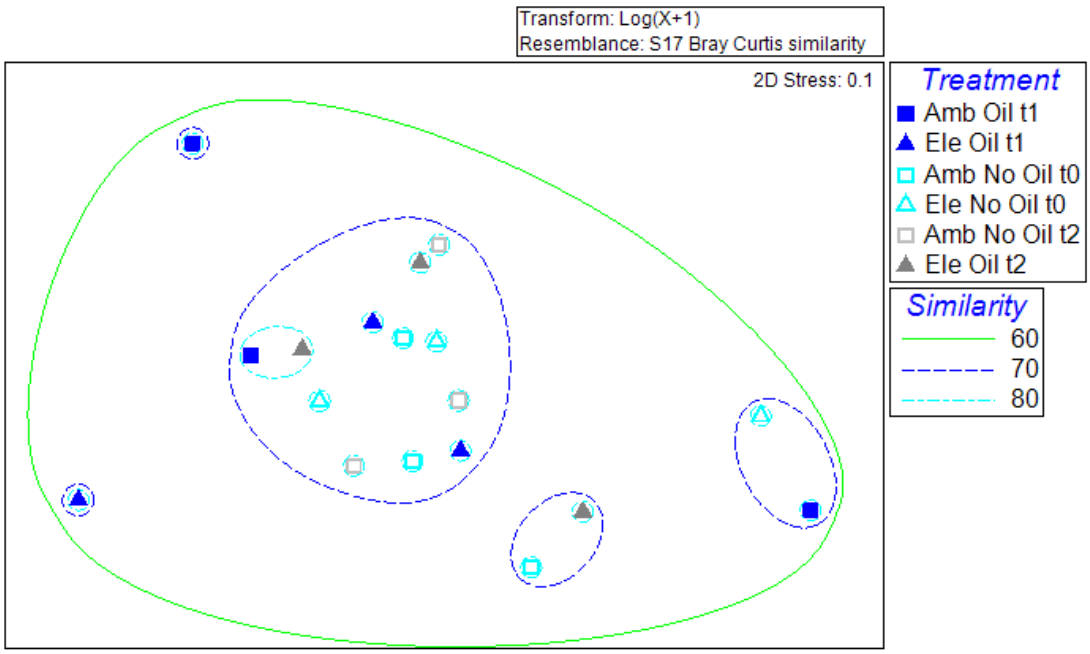


Figure 3.4. NMDS plots based on Bray Curtis similarity index of the bacterial community associated with *E. huxleyi* in microcosms treated with ambient/elevated CO_2, prior to oil enrichment (t0), at four timepoints (t0; day 5/ prior to oil enrichment, t1; day 6/one day after oil exposure, t2; day 21/16 days after oil exposure). Clusters based on log (X + 1) Bray-Curtis similarity index (%) are indicated by coloured ellipses.

The most abundant (mean % ± SE) taxa from the total bacterial community associated to *E. huxleyi* in ambient treated microcosms at t0 were *Marinobacter* (29% ± 1), followed by unclassified *Gammaproteobacteria* (11% ± 5), *Methylobacterium* (8% ± 2), unclassified *Alphaproteobacteria* (6% ± 2), unclassified bacteria (6% ± 3), and *Sphingomonas* (5% ± 1; Table 3.1). Less abundant (< 5%) taxa in ambient treated microcosms at t0 includes *Maricaulis* (4% ± 1), *Balneola* (4% ± 1), unclassified *Rhodobacteraceae* (4% ± 1), *Sulfitobacter* (3% ± 1), unclassified *Alteromonadaceae* (3% ± 3), *Streptococcus* (3% ± 1), *Gracilimonas* (2% ± 0), *Methylophaga* (2% ± 0), *Prevotella* (2% ± 0), *Celeribacter* (2% ± 0), *Alteromonas* (2% ± 0), *Halomonas* (2% ± 0), *Corynebacterium* (1% ± 1), and *Veillonella*. (1% ± 1; Table 3.1).

Log2 median ratio comparison of ambient treated microbiota of *E. huxleyi* compared to elevated CO_2 treated microbiota of *E. huxleyi* shows higher ratio of *Marinobacter* and *Methylobacterium* under ambient CO_2 conditions (Figure 3.6). Loss of *Marinobacter* from 29% ± 1 to 15% ± 4 (0 – 19%) and *Methylobacterium* (1 -3%) were correlated with mean percentage of taxa in respective bacterial community at t0 (Table 3.1). No taxa were enriched at elevated CO_2 prior to oil enrichment (t0) compared to ambient treated microcosms prior to oil enrichment (t0). Bacterial community acclimated to elevated CO_2 were dominated by *Marinobacter* (15 % ± 4), unclassified bacteria (13% ± 8), *Methylobacterium* (11% ± 4), *Sphingomonas* (8% ± 2), unclassified *Halomonadaceae* (6% ± 6), unclassified *Rhodobacteraceae* (5% ± 4), *Halomonas* (5% ± 2), *and Balneola* (5% ± 1).

Oil enrichment elicits a different response in community succession in ambient and elevated CO_2 treated microcosms. Enrichment of *Sphingomonas* and unclassified bacteria was seen when bacterial community treated in ambient CO_2 (t0) was exposed to crude oil after 1 day (t1) while *Marinobacter* was negatively affected (Figure 3.6). *Sphingomonas* increased by 0 – 2%, unclassified bacteria increased by 0 – 18%, and *Marinobacter* reduced by 10 – 18% (Table 3.1). By contrast, unclassified *Gammaproteobacteria* and *Marinobacter* were enriched when elevated CO_2 treated microcosm (t0) were enriched with crude oil (t1), and no taxa were negatively affected (Figure 3.6). Unclassified *Gammaproteobacteria* was enriched by 0 – 7% while *Marinobacter* was enriched by 1 -21% (Table 3.1).

Comparison between CO_2 treatments in response to oil enrichment at t1 shows that taxa from the class *Alphaproteobacteria: Methylobacterium, and Sphingomonas*, in addition to unclassified bacteria were more prevalent under ambient/oil-enriched (t1) treatment compared to elevated/oil-enriched treatment (t1) while taxa from the class *Gammaproteobacteria*: *Marinobacter* and unclassified *Gammaproteobacteria* were more successful at elevated/oil-enriched treated microcosms (Figure 3.6).

Ambient/oil-enriched bacterial community were dominated by unclassified bacteria (18% ± 9), *Marinobacter* (15% ± 3), *Methylobacterium* (11% ± 4), unclassified *Alphaproteobacteria* (8% ± 5), unclassified *Rhodobacteraceae* (7% ± 1), and *Sphingomonas* (6% ± 2; Table 3.1). Elevated/oil enriched bacterial community were dominated by *Marinobacter* (24% ± 8), unclassified bacteria (9% ± 6), *Prevotella* (8% ± 7), unclassified *Gammaproteobacteria* (7% ± 3), *Streptococcus* (7% ± 4), and *Methylobacterium* (6% ± 3; Table 3.1).

Following bacterial community succession of elevated/oil-enriched at t1, unclassified bacteria, *Marinobacter* and unclassified *Gammaproteobacteria* were further enriched in elevated/oil-enriched microcosms at t2 (Figure 3.6). The taxon *Alphaproteobacteria* were negatively affected at t2 when compared to elevated/oil-enriched treated microcosms (Figure 3.6). The same enrichment of taxa were seen in elevated/oil-enriched microcosm at t2 when compared to control treatment; ambient/without oil-enriched microcosm at t2, while *Methylobacterium* from the class Alphaproteobacteria were seen enriched in ambient/without oil-enriched microcosm compared to elevated/oil-enriched microcosms at t2 (Figure 3.6).

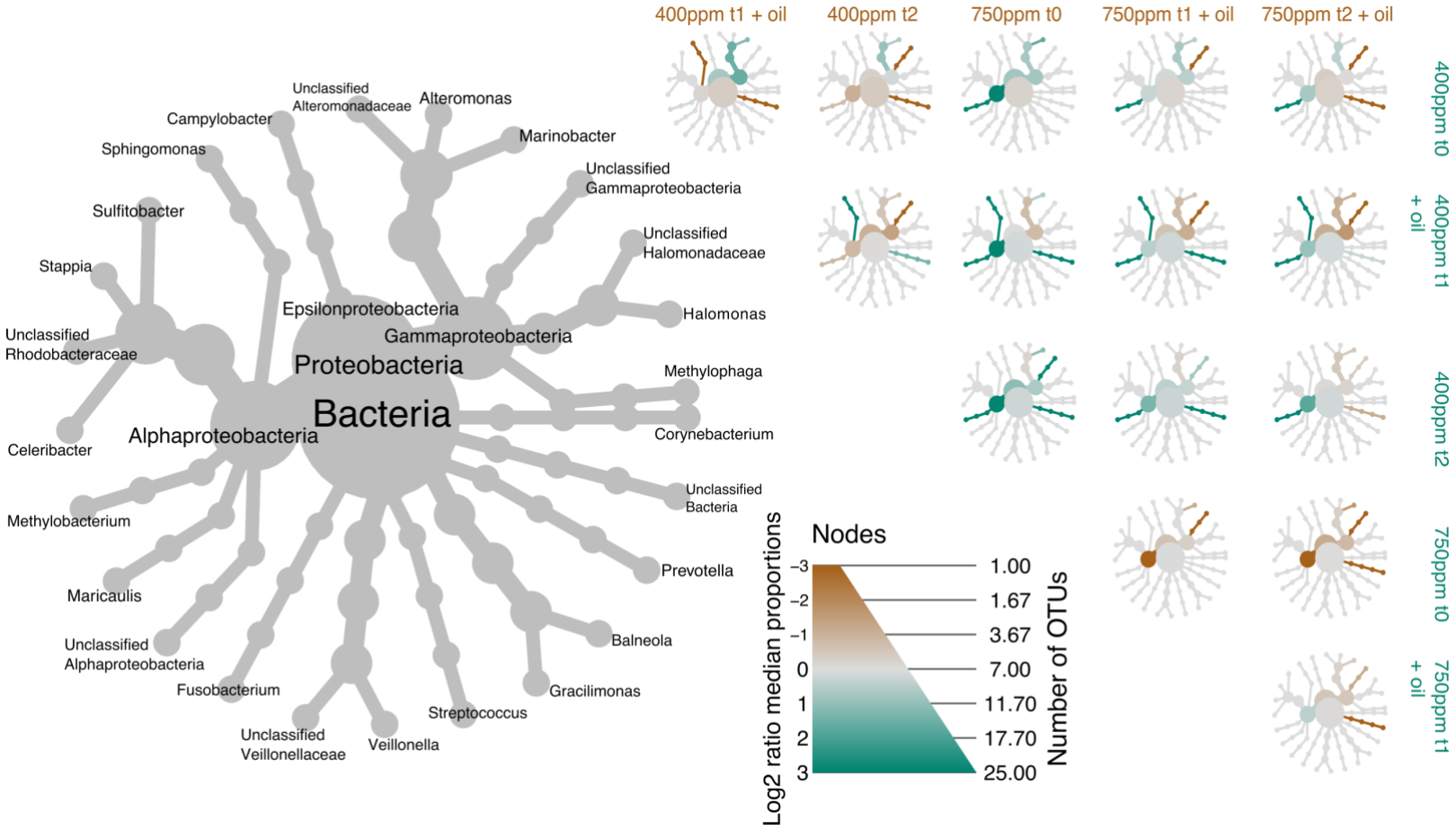


Figure 3.5. Heat-tree plot of bacterial community associated with *E. huxleyi* in different CO_2 and oil enrichment treatments. Larger grey tree represents taxon labelled key of the smaller heat trees showing pairwise comparison between treatments. Diameter of nodes shows qualitative number of OTU of each taxon. Colour intensity of smaller trees (green or brown) corresponds to significantly abundant taxons between treatments determined with Wilcox rank-sum test followed by Benjamini Hochberg (FDR) correction for multiple comparisons. Treatments correspond to CO2 acclimation at 400ppm (ambient) or 750ppm(elevated) at different timepoints. T0 was sampled at day 5 prior to oil enrichment, t1; day 12 without oil or 7 days after oil enrichment, and t2; day 19 without oil enrichment or 14 days after oil enrichment.

Table 3.1. Average relative abundance (%) ± standard error of *E. huxleyi* associated bacterial OTU count between 400 ppm (ambient) and elevated CO2 (750 ppm) and enrichment/absence of crude oil (+/-) across timepoints (t0; prior to oil enrichment (day 4), t1; 7 days after oil enrichment (day 12), t2; 14 days after oil enrichment/day 19). Relative abundance (%) were rounded to nearest number and OTU's were grouped into respective phyla.

Timepoint	**T0**		**T1**		**T2**	
CO_2	**400 ppm**	**750 ppm**	**400 ppm**	**750 ppm**	**400 ppm**	**750 ppm**
Oil enrichment	**-**	**-**	**+**	**+**	**-**	**+**
No. of OTU's	**89 ± 21**	**59 ± 25**	**45 ± 14**	**95 ± 32**	**52 ± 39**	**85±20**
Unclassified Bacteria	6 ± 3	13± 8	18 ± 9	9 ± 6	16 ± 10	8 ± 2
Actinobacteria (Phyla)						
Corynebacterium	1 ± 1	2 ± 1	4 ± 2	2 ± 1	2 ± 2	4 ± 1
Bacteroidetes (Phyla)						
Prevotella	2 ± <1	1 ± 1	1 ± 1	8 ± 7	<1 ± <1	0 ± 0
Balneola	4 ± 1	5 ± 1	3 ± 1	3 ± 2	5 ± 2	3 ± <1
Gracilimonas	2 ± <1	0 ± 0	<1 ± <1	4 ± 2	1 ± 1	<1±<1
Firmicutes (Phyla)						
Streptococcus	3 ± 1	1 ± 1	0 ± 0	7 ± 4	1 ± 1	1 ± <1
Veillonella	1 ± 1	0 ± 0	<1 ± <1	5 ± 3	2 ± <1	1 ± 1
Unclassified *Veillonellaceae*	0 ± 0	0 ± 0	0 ± 0	3 ± 2	0 ± 0	0 ± 0
Fusobacteria (Phyla)						
Fusobacterium	0 ± 0	1 ± 1	0 ± 0	3 ± 2	0 ± 0	1 ± 1
Proteobacteria (Phyla)						
Alphaproteobacteria (Class)						
Unclassified *Alphaproteobacteria*	6 ± 2	4 ± 1	8 ± 5	3 ± 1	4 ± 1	5 ± 3
Maricaulis	4 ± 1	4 ± 1	2 ± 1	4 ± 2	4 ± 2	2 ± 1
Methylobacterium	8 ± 2	11 ± 4	11 ± 4	6 ± 3	10 ± 3	6 ± <1
Celeribacter	2 ± <1	2 ± 1	2 ± 1	1 ± <1	3 ± 1	2 ± <1
Unclassified *Rhodobacteraceae*	4 ± 1	5 ± 4	7 ± 1	3 ± 1	5± 4	4 ± 3
Stappia	<1 ± <1	<1 ± <1	1 ± <1	3 ± 2	1 ± 1	1 ± 1

Continued Table 3.1. part 2/2

Timepoint	**T0**		**T1**		**T2**	
CO_2	**400 ppm**	**750 ppm**	**400 ppm**	**750 ppm**	**400 ppm**	**750 ppm**
Oil enrichment	**-**	**-**	**+**	**+**	**-**	**+**
No. of OTU's	**89 ± 21**	**59 ± 25**	**45 ± 14**	**95 ± 32**	**52 ± 39**	**85±20**
Sulfitobacter	3 ± 1	1 ± 1	2 ± 1	4 ± 2	4 ± 1	3 ± 1
Sphingomonas	5 ± 1	8 ± 2	6 ± 2	4 ± 1	6 ± 2	3 ± 1
Gammaproteobacteria (Class)						
Unclassified *Alteromonadaceae*	3 ± 3	4 ± 3	2 ± 2	2 ± 2	<1 ± <1	3 ± 3
Alteromonas	2 ± 0	3 ± 2	2 ± 1	1 ± <1	2 ± <1	3 ± 1
Marinobacter	29 ± 1	15 ± 4	15 ± 3	24 ± 8	20 ± 4	22 ± 5
Unclassified *Gammaproteobacteria*	11 ± 5	4 ± 1	5 ± 2	7 ± 3	8 ± 5	8 ± 4
Unclassified *Halomonadaceae*	0 ± 0	6 ± 6	5 ± 5	0 ± 0	0 ± 0	5 ± 5
Halomonas	2 ± <1	5 ± 2	3 ± 1	1 ± <1	2 ± 1	3 ± 1
Methylophaga	2 ± <1	4 ± 1	4 ± 2	2 ± 1	4 ± 1	3 ± 1
Epsilonproteobacteria (Class)						
Campylobacter	0 ± 0	0 ± 0	<1 ± <1	2 ± 2	1 ± 1	0 ± 0

3.4.3. Hydrocarbon profile

Gas chromatography analysis of hydrocarbon compounds inoculated with *E. huxleyi* and its associated bacterial community treated with ambient/ elevated CO_2 were compared with acid-killed *E. huxleyi* and microbiota to identify degradation of crude oil from biotic factors and not by weathering (Figure 3.6). Aliphatic compounds were analysed between nC8 to nC43. nC8 to nC10 were not detected in both acid-killed and inoculated treatments. Ratios of aliphatic compounds (nC17/pristine and nC18/ phytane) are common indices used as biomarkers to identify biodegradation of crude oil (Xiong *et al.*, 2015).

Biodegradation was determined in both CO_2 treated microcosms after 14 days of crude oil exposure to *E. huxleyi* and its microbiota since the decrease in nC17/pristine ratios and nC18/phytane ratios were significantly different in ambient ($p < 0.0001$) and

elevated ($p < 0.0001$) treated microcosms compared to the respective killed controls (Figure 3.5). However, mean difference of 0.01 of nC17/pristane ratio and 0.0233 of nC18/phytane ratio between live samples treated under ambient CO_2 compared to elevated CO_2 were not significantly different ($p = 0.9874$ and $p = 0.8695$ respectively).

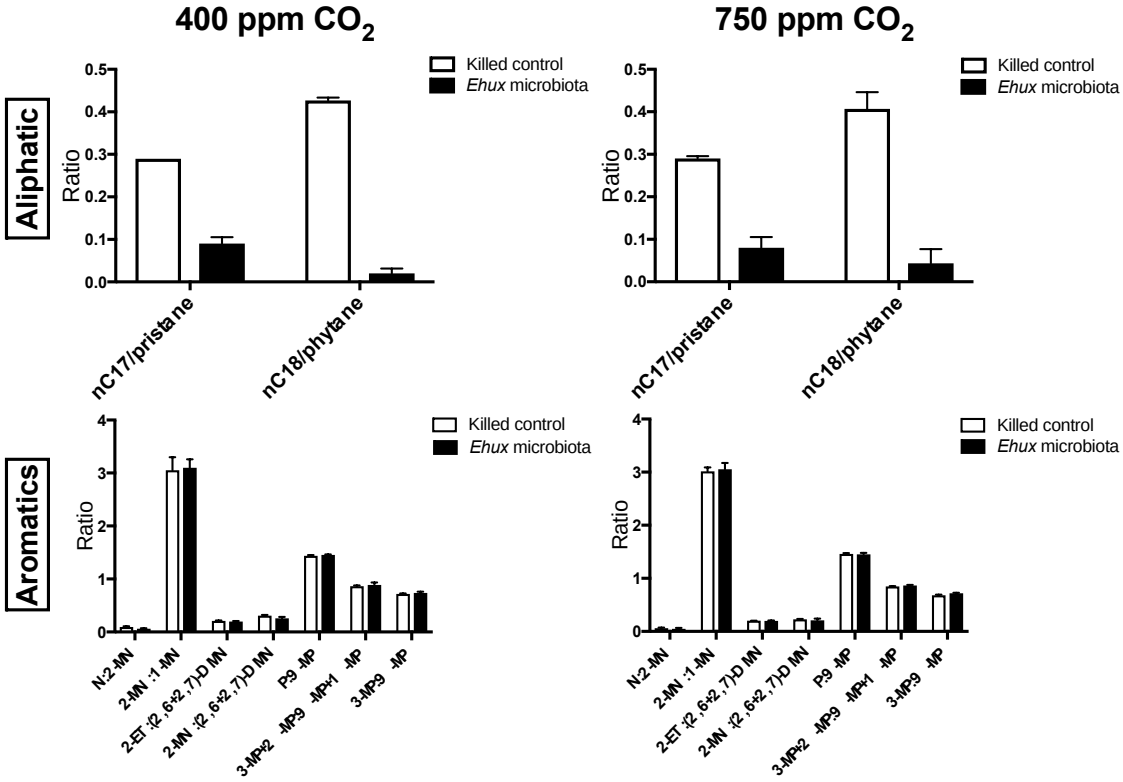


Figure 3.6. Differences in hydrocarbon ratios comparing live treatments of *E. huxleyi* associated bacterial community (solid bars; *Ehux* microbiota) to their respective acidified controls (open bars; killed controls) for parameters indicative of biodegradation: nC17/pristine, nC18/phytane, naphthalene/2-methylnaphthalene (N:2-MN), 2-methylnaphthalene/1-methylnaphthalene (2-MN;1-MN) 2-ethylnaphthalene/2,6+2,7-dimethylnapthalene (2-ET:(2,6+2,7)-DMN), phenanthrene/9-methylphenanthrene (P:9-MP), 3-methylphenanthrene+2methylphenanthrene/9-methylphenanthrene+1-methylphenanthrene (3-MP+2-MP:9-MP+1-MP), 3-methylphenanthrene/9-methylphenanthrene (3-MP:9-MP). Values are averages of triplicate incubations with error bars showing standard error of measurement (SEM).

Ratios of aromatic compounds: naphthalene (N), methylnapthalene (MN), dimethylnaphthalene (DMN), ethylnaphthalene (ET), C3-alkylnaphthalene (TMN), phenanthrene (P), methylphenanthrene (MP), dimethylphenanthrene (DMP), ethylphenanthrene (ET), and C3-alkylphenanthrene (TMP) were analysed but no significant differences were detected in all aromatic compounds treated with live samples

compared to acidified controls in both ambient and elevated CO_2 concentrations (Figure 3.5).

3.3.4. Carbonate chemistry of f/2 amended seawater media in microcosms

Linear regression of daily average pCO_2 concentration supplied by mixing ambient air with CO_2 from compressed gas tank shows the achieved pCO_2 level for elevated CO_2 condition is 740 ppm, only 10 ppm lower from the targeted elevated CO_2 predicted for the year 2100 (Figure 3.7). Two-way ANOVA and Tukey's multiple comparison test of pH of seawater media with f/2 nutrient amendments with/without *E. huxleyi* and its associated bacterial community were not significantly different on day 1, day 2, day 3, and day 4 ($p > 0.05$). Prior to oil enrichment, mean difference of 0.57 between inoculated microcosms treated with ambient CO_2 were significantly different from inoculated microcosms treated with elevated CO_2 ($p = 0.0107$; Figure 3.6). When crude oil was enriched in microcosms, pH of seawater in microcosms in all treatments decreased.

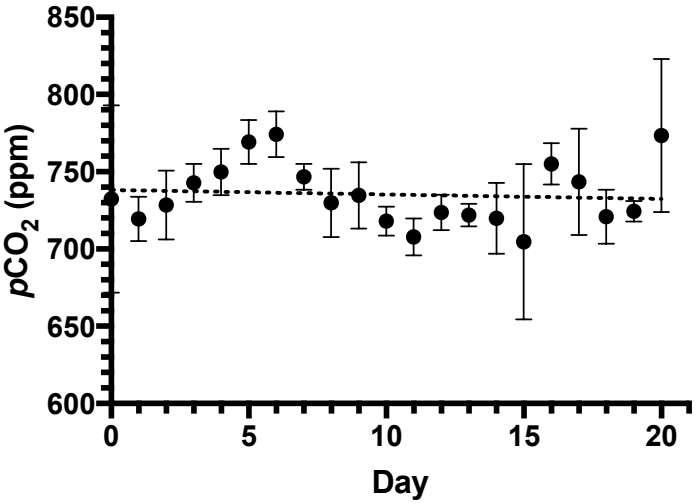


Figure 3.7. Daily average of CO_2 concentration (ppm) supplied to microcosms exposed to elevated pCO_2 conditions with linear regression (dotted lines) showing level of elevated pCO_2 concentration exposed to microcosms. Error bars shows standard deviation of pCO_2 per day.

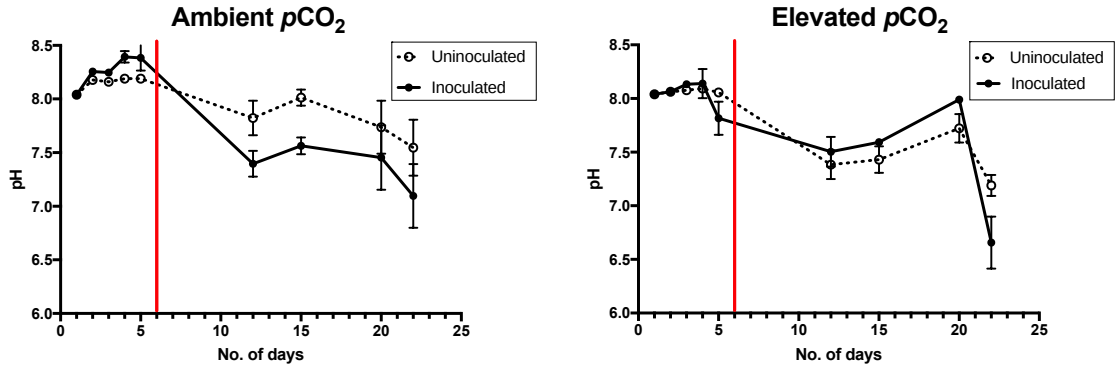


Figure 3.8. pH levels of seawater in microcosms with/without *E. huxleyi* and its associated bacterial community in ambient (400 ppm) and elevated (750 ppm) CO_2 conentrations. Microcosms enriched with crude oil after day 5 (red line). Microcosms without *E. huxleyi* and its microbiota (uninoculated; open circle) were compared to microcosms with *E. huxleyi* microbiota (inoculated; closed circle). Values are averages of triplicate incubations with error bars showing standard error of measurement (SEM).

Two-way ANOVA and Tukey's multiple comparison test shows no significant differences of pH between all treatments on day 12 ($p > 0.05$). Mean difference of 0.5833 pH units between uninoculated microcosms treated in ambient CO_2 and uninoculated microcosms treated in elevated CO_2 conditions were significantly different ($p = 0.0081$) on day 15, or 10 days after crude oil exposure (Figure 3.8). After 15 days of crude oil exposure (day 20), microcosms inoculated with *E. huxleyi* and its microbiota treated in ambient and elevated CO_2 showed a mean difference of 0.54 pH units and was significantly different ($p = 0.0174$). On the final day of the experiment (day 21), pH of ambient/without *E. huxleyi* microbiota were significantly different from elevated CO_2/with *E. huxleyi* microbiota ($p < 0.0001$). In addition, pH of elevated CO_2/with *E. huxleyi* microbiota were also significantly different to elevated CO_2/ without *E. huxleyi* microbiota ($p = 0.0183$) on day 21. Ambient/inoculated with *E. huxleyi* microbiota were not significantly different from elevated/CO_2 with *E. huxleyi* microbiota ($p > 0.05$) after 16 days of crude oil enrichment (day 21).

Total dissolved inorganic carbon (C_T) and pH total scale (pH_T) measured for each microcosm were used to calculate other carbonate chemistry parameters such as pCO_2 in the seawater (µatm), total alkalinity (A_T), and saturation of calcite ($\Omega_{Calcite}$) were found inconsistent with achieved pCO_2 levels as a result of calculating linear regression of pCO_2 levels supplied to microcosms that were monitored and measured per second throughout the experiment (Figure 3.7). Although the machines were calibrated with reference seawater, the error was introduced when salinity of samples was measured only in the

beginning of experiment and error in calibration of probe used for determination of pH_T. Changes in temperature and evaporation during experiment and storage of samples prior to DIC measurements may have altered salinity thus affecting calculated values of carbonate chemistry.

3.4. Discussion

3.4.1. Impact of ocean acidification and oil enrichment on E. huxleyi

E. huxleyi maintained in f/2 supplemented seawater resulted in a culture of high density reaching more than 1 x 10^6 cells per litre or more than 13.8 of Ln of cells per litre by day 5 in both CO_2 conditions (Figure 3.2; Head *et al.*, 1998; Tyrrell and Merico, 2004). Dense cultures increases water albedo or reflectance, mutual shading, and by-product formation (Tyrrell and Merico, 2004; Jakob, Weggenmann and Posten, 2018). The microcosm setup minimises self-shading as the placement of light source that was parallel to microcosm (Figure 2.3) increases surface area for light to penetrate through the microcosm and availability of light to pass through medium does not decrease with depth (Posten and Rosello-Sastre, 2011). By product formation in dense *E. huxleyi* cultures include metabolic storage metabolites like alkenones and mannitol, climate-influencing metabolites such as methane and DMS, and structure forming metabolites such as coccolith AP polysaccharide (Obata *et al.*, 2013; Tsuji *et al.*, 2015). Although quantification of by-products, or metabolites in both CO_2 conditions were not carried out, it is likely that the production or release of these metabolites under high cell density largely influences the bacterial community associated to *E. huxleyi.* Furthermore, another methodological limitation encountered in this study was the lack of quantification of nutrients such as N and P in different CO_2 conditions throughout the experiment. Although nutrient levels in f/2 media does not reflect natural oligotrophic conditions of the open ocean, rate of uptake of important nutrients such as C, N, and P influence the dynamic interaction between microalgal host and associated bacterial community.

Decreasing pH by increasing CO_2 concentration have been shown to reduce growth rate of *E. huxleyi* due to metabolic cost of maintaining intracellular pH (Nimer, Brownlee and Merrett, 1994; Fukuda, Suzuki and Shiraiwa, 2014). However, similar to Fukuda, Suzuki and Shiraiwa, (2014), this study reported no significant differences were seen from chl *a* analysis by fluorometric analysis of *E. huxleyi* under elevated CO_2. Bach *et al.*, (2015) proposed the increase in inorganic substrate such as bicarbonate compensates the dominating inhibiting factor of protons (H+) to calcification rates during ocean acidification conditions. This was shown in the first 4 days of culturing, where chl

a concentration in both CO_2 treatments were similar. Chl *a* concentration was significantly higher in elevated CO_2 treated microcosms compared to ambient treated microcosm on day 5 as inorganic carbon is no longer the limiting factor in elevated CO_2 conditions. In ambient conditions, autotrophy and the production of organic molecules from photosynthesis was limited by ambient CO_2 concentrations. When CO_2 levels were increased to 750 ppm, the capacity to produce organic molecules from photosynthesis is higher thus allows higher chl *a* concentration in elevated CO_2 conditions. CO_2 is a limiting factor in photosynthesis; therefore, the availability of higher CO_2 concentrations can be utitlised to compensate for the metabolic stress of maintaining intracellular pH by increasing chl *a* concentration. Mesocosm experiments show higher concentration of chl *a* during exponential phase in elevated CO_2 treatment (Riebesell *et al.*, 2013; Liu *et al.*, 2017).

One day after microcosms were exposed to crude oil, chl *a* concentration continued to increase in ambient CO_2 treated microcosms and were still detected after 9 days of crude oil exposure. Chl *a* in ambient/oil-enriched microcosms were relatively higher than in control experiments where *E. huxleyi* was grown in ambient/without oil enrichment. Negative response of *E. huxleyi* to crude oil enrichment under elevated conditions were immediate as chl *a* concentration stopped exponential growth phase and entered stationary phase. This was followed by death of the population seen on day 12, only 7 days after oil exposure. *E. huxleyi* population death under elevated CO_2/oil-enriched treatment were 8 days earlier than in ambient/oil-enriched treatment.

It is hypothesised that under ambient CO_2, *E. huxleyi* was able to acclimate better to the crude oil exposure by adopting the 'Cheshire Cat' escape strategy where morphological and structural changes that occur during the haploid life stage allows avoidance of chemical and physical stressors imposed by crude oil (Frada *et al.*, 2008). When *E. huxleyi* population is predominantly in its haploid stage, its motility by flagellation and lack of mineralised scales allows evasion of physical, chemical, and biotic stress (Valero *et al.*, 1992; Thornber, 2006; Frada *et al.*, 2008). Whereas when crude oil was exposed to *E. huxleyi* under elevated CO_2 conditions, the impact was aggravated as non-motile, mineralised population in the diploid state are more susceptible to negative impact of chemical and physical properties of crude oil. However, this hypothesis was not proven as no evidence of cell morphology were collected. It is assumed that the diploid phase is prevalent in carbon-rich seawater and promotes phytoplankton blooms as described in a previous study (Rokitta *et al.*, 2014).

Furthermore, the opposing response of *E. huxleyi* to oil enrichment under different CO_2 conditions is likely due to the microbiota-host relationship prior to oil enrichment.

3.4.2. Impact of ocean acidification and oil enrichment on bacterial community

Ordinance of bacterial community of *E. huxleyi* microbiota was not significantly different when acclimated under 750 ppm and when exposed to crude oil. Bacterial community were only 40% dissimilar from ambient/elevated CO_2 treatment and crude oil exposure up to 16 days (Figure 3.4). The dissimilarity between bacterial community was observed in relative abundance of taxa between treatments (Figure 3.4). Difficulty in extraction and analysis of molecular samples, in addition to bias of amplification and high-throughput sequencing may be omitted, distorted and/or misrepresented thus does not perfectly capture the diversity of bacteria associated with *E. huxleyi* (Acinas *et al.*, 2005; Hong *et al.*, 2009; Lee *et al.*, 2012; Pinto and Raskin, 2012; Logares *et al.*, 2014). However primers are continuously altered and the primers used in this study is the best standard practice proposed by Earth Microbiome Project thus allows for comparison and recognised bias for underrepresentation of SAR11 clad have greatly increased (Apprill *et al.*, 2015; Caporaso *et al.*, 2018). In addition, bacterial cell counts were not obtained to correlate absolute cell count with OTU counts detected by high-throughput sequencing. Therefore, relative abundance of taxa (%) from each sample only allows for comparison of community structure and not absolute bacterial counts between treatments.

Dominance of *Marinobacter* and *Methylobacterium* in enriched f/2 media and high cell abundance of *E. huxleyi* can be associated with metabolite production of *E. huxleyi* bloom. Abundance of bioavailable metabolites such as mannitol and methane produced by *E. huxleyi* in high concentration (Obata *et al.*, 2013; Tsuji *et al.*, 2015) was likely a dominant selection factor for both genus of bacteria even after enrichment of crude oil. In ambient conditions prior to oil enrichment, median log2 ratio comparison of bacterial community with elevated CO_2 treated microcosms show *Marinobacter* and *Methylobacterium* were more prevalent under ambient CO_2 than elevated CO_2 conditions prior to oil enrichment. Decrease in mean percentage of *Marinobacter* by 0-19% (29 ± 1% to 15 ± 4%; Table 3.1) correlated to relatively lower chl *a* concentration however, analysis of nutrient levels was not carried out to prove whether competition or abundance or lack of bioavailable nutrients were the cause for reduced relative abundance of *Marinobacter* in OA conditions. Although primary production increases production of organic carbon, photosynthesis utilises micronutrients such as iron that are co-limiting growth factors for heterotrophic bacteria (Tortell *et al.*, 1999; Amin *et al.*, 2012;

Schoffman *et al.*, 2016). Furthermore, enrichment of *Marinobacter* after oil-enrichment under elevated CO_2 postulates relative abundance of *Marinobacter* was nutrient-dependent and not directly related to elevated CO_2 conditions.

Mean percentage of *Methylobacterium* from the total bacterial community in elevated CO_2 conditions decreased by 1-3%. *Methylobacterium* continued to thrive under ambient/oil-enriched treated microcosms at t1 but not in elevated/oil-enriched CO_2 (Table 3.1). It can be concluded that *Methylobacterium* was negatively affected by elevated CO_2 conditions. Higher mean percentage of *Marinobacter* and *Methylabacterium* in ambient CO_2 treated microcosms mitigate the impact of crude oil enrichment on day 5. *Marinobacter* is a genus known to utilize aliphatic and polycyclic aromatic hydrocarbons as a carbon source (Vila *et al.*, 2010; Gutierrez, Singleton, *et al.*, 2013; Kappell *et al.*, 2014). *Methylobacterium* is a methylotrophic genera capable of utilizing single carbon compounds such as methanol and methane (Green, 2006; Mukherjee *et al.*, 2017).

After enrichment of crude oil, *Marinobacter* and unclassified *Gammaproteobacteria* were more enriched under elevated CO_2 treatments compared to ambient CO_2 at t1. Further enrichment of *Marinobacter* and unclassified *Gammaproteobacteria* were observed under elevated CO_2/oil-enriched conditions at t2. The availability of compounds from crude oil that was potentially utilised by both taxa have supplemented the growth of their population up to 16 days after crude oil enrichment. In addition to enrichment of *Methylobacterium*, *Sphingomonas*, and unclassified bacteria were enriched under ambient/oil-enriched conditions. It is very likely that *Sphingomonas* and unclassified bacteria outcompeted *Marinobacter* under ambient/oil-enriched conditions. *Sphingomonas* are metabolically diverse and have been isolated from oligotrophic conditions, and have been identified as hydrocarbon degraders (Balkwill, Fredrickson and Romine, 2006). Instead of a lipopolysaccharide cell envelope, *Sphingomonas* has glycosphingolipids that increases their hydrophobicity and allows for contact and degradation of polycyclic aromatic hydrocarbons (PAH) (Kawahara *et al.*, 2001; Balkwill, Fredrickson and Romine, 2006). Both *Methylobacterium* and *Sphingomonas* belong to the class *Alphaproteobacteria*, and their decrease in relative abundance under elevated CO_2 supports other studies that found negative impacts of ocean acidification on *Alphaproteobacteria* (Witt *et al.*, 2011; Hassenrück *et al.*, 2016).

Other hydrocarbonoclastic genus such as *Halomonas*, and *Alteromonas* (Gutierrez, Singleton, *et al.*, 2013) were present but not affected by CO_2 treatments. Green et al., (2015) described other hydrocarbon degrading genera found associated to *E. huxleyi* of the same strain such as *Marivita, Hoeflea, Balneola, Arenibacter,*

Marinoscillum, and *Thalassospira*. These genera were identified in the preliminary analysis; however, their abundance was not significant to be included in the heat tree matrix (OTU count less than 5). The isolation of the aforementioned genus is likely due to selection bias of bacterial cultivation by plating in ONR7a plates amended with n-hexadecane filter paper described by Green et al. (2015). *Phaeobacteria inhibens*, a roseobacter widely reported for its close interaction and regulation of *E. huxleyi* blooms (Seyedsayamdost *et al.*, 2011; Segev *et al.*, 2016; Bramucci *et al.*, 2018) were not identified to be associated with *E. huxleyi* strain 920/8 provided by CCAP used in this experiment.

Seven unclassified taxa were identified in this study; 1) unclassified bacterium that was more abundant in ambient condition with oil enrichment, 2) unclassified *Alphaproteobacteria*, 3) unclassified *Gammaproteobacteria*, 4) unclassified *Alteromonodaceae*, 5) unclassified *Veillonellaceae*, 6) unclassified *Hallomonadaceae* and 7) unclassified *Rhodobacteriaceae*. Out of the seven, only two were affected by CO_2 treatments; unclassified *Gammaproteobacteria* showed higher abundance in elevated CO_2 conditions with oil enrichment. This suggests enrichment of potentially novel strains that are prevalent in biodegradation or tolerance to crude oil in higher atmospheric CO_2 conditions, while the abundance of unclassified bacteria in ambient CO_2, enriched oil, suggests potential loss of novel bacteria with unknown environmental functions due to sensitivity to OA stress.

3.4.3. Biodegradation of crude oil

Biodegradation of aliphatic compounds was identified in both ambient and elevated conditions at the end of the experiment by comparing nC17/pristane and nC18/phytane ratios of acid-killed samples with live inoculated samples (Figure 3.6). Despite shifts in microbial population dynamics due to elevated CO_2 conditions (Figure 3.5), aliphatic hydrocarbon degradation was not compromised by elevated CO_2 treatment. nC8, nC9, and nC10 were not found in all treatments (Figure 3.6) and are likely absent due to weathering of compounds after 16 days of crude oil exposure in microcosms.

Aromatic compound such as naphthalene (N), methylnapthalene (MN), dimethylnaphthalene (DMN), ethylnaphthalene (ET), C3-alkylnaphthalene (TMN), phenanthrene (P), methylphenanthrene (MP), dimethylphenanthrene (DMP), ethylphenanthrene (ET), and C3-alkylphenanthrene (TMP) were not biodegraded in both CO_2 treated microcosms after 16 days of crude oil exposure. It is very likely that microbial community associated to *E. huxleyi* did not have the metabolic potential to degrade previously mentioned aromatic compounds or limited by co-availability of other

nutrients. Elimination of pollutants are also dependent on the action of several microorganisms performing additional reactions (McGenity *et al.*, 2012).

3.4.4. Carbonate chemistry of f/2 amended seawater media in microcosms

pH was higher when *E. huxleyi* was inoculated in ambient treated CO_2 prior to oil enrichment compared to uninoculated control and elevated CO_2 treated *E. huxleyi*. The increased levels of hydrogen ions measured by pH throughout active growth of microalga is attributed to active transport of H^+ for the maintenance of intracellular pH during consumption of ATP and nitrate uptake (Solovchenko and Khozin-Goldberg, 2013). This expected increase in pH from *E. huxleyi* was counteracted in elevated CO_2 treatments by acidification of media. On day 5, prior to oil enrichment and at peak chl *a* concentration, pH levels of *E. huxleyi* and elevated CO_2 media dropped lower than uninoculated controls. Fukuda *et al.*, (2011) states that when photosynthetic activity was higher than respiration, calcification and the consequent release of CO_2 into the media reduces pH levels of media. The presence of oil reduces pH levels of all treatments and significantly increases the difference of C_T between aerated treatments at the end of the experiment. The oil prevents outgassing of CO_2 from its aqueous phase, as total CO_2 builds up due to direct bubbling of CO_2 treatments into the water column of the microcosms. Despite significantly higher levels of total dissolved CO_2 in oil enriched microcosms, pH levels were not significantly different suggesting buffering capacity of the seawater media in both aeration treatments.

3.5. Conclusion

Findings from this chapter shows that although growth rate of *E. huxleyi* were not affected by projected future conditions (750 ppm), chl *a* concentration was much higher. The response of *E. huxleyi* blooms to crude oil pollution under 750 ppm indicates impacts of oil pollution were exacerbated by ocean acidification. Despite the vulnerability of the host, the microbiota of *E. huxleyi* was resilient to oil enrichment under elevated CO_2 conditions. *Marinobacter* was the dominant bacteria associated to *E. huxleyi* prior to oil-enrichment in both ambient and elevated CO_2 conditions, and in elevated CO_2/oil-enriched conditions up to 16 days after oil exposure. Only under ambient/oil-enriched conditions were *Marinobacter* overtaken by unclassified bacteria as the dominant taxa. Significant changes in population dynamics after oil enrichment between CO_2 treatments where relative abundance of *Sphingomonas* and *Methylobacterium* from the class

Alphaproteobacteria were reduced, did not significantly affect degradation of pristane and phytane from crude oil.

Laboratory strain *E. huxleyi* grown in f/2 enriched media reached cell density comparable to blooms, however, do not reflect natural occurring coccolithophore blooms and associated bacterial diversity. Despite the limitation of methodology and relevance to real-world scenario, the chl *a* response to elevated CO_2 and oil enrichment hints at a compromised defense to toxic components of crude oil under elevated CO_2 condition potentially attributed to dynamic relationship between *E. huxleyi* and its associated bacterial community. Changes in bacterial population, though small, should not be taken lightly as it is crucial to host survival. Recovery of an ecosystem from oil pollution under projected future ocean condition depends not just on the potential for hydrocarbon degradation, but also on the microalgal host survival and primary production. Therefore, in a natural community of phytoplankton, where multiple species of microalgae and their microbiota co-exist and interact with each other, how will they respond to crude oil enrichment under projected ocean acidification conditions?

Chapter 4: Impacts of ocean acidification and crude oil pollution on spring bacterioplankton community (Faroe-Shetland Channel)

4.1. Introduction

In this chapter, the response of natural microalgal-bacterial community to ocean acidification and oil pollution will be investigated using the same experimental setup and treatments as described in Chapter 3. Intraspecies interactions between major groups of bloom-forming microalgae, in addition to their associated bacterial communities, introduces a more realistic response of microbial community to oil pollution in future ocean conditions. The effects of elevated CO_2 has been identified as a cofactor of earlier seasonal blooms of microalgae in addition to changing weather patterns (Peeters *et al.*, 2007; Mignot, Ferrari and Claustre, 2018). OA studies have shown dissolved CO_2-enrichment caused a fertilising effect on microalgae in resource limited conditions, and have varying degrees of negative impacts on survival, growth, calcification, and reproduction of marine organisms (Kroeker *et al.*, 2011; Paul, Matthiessen and Sommer, 2015).

The North Atlantic Ocean is an ice-free open water region exposed to extreme light-cycle shifts during winter and summer (Sundby, Drinkwater and Kjesbu, 2016). Phytoplankton blooms in the northern most region of the Atlantic have been described as highly active, with the most dramatic seasonal increase of phytoplankton on a global scale (Mignot, Ferrari and Claustre, 2018). Quite recently, a long-term *in-situ* study using multiple bio-optical sensors attached to floats that enables vertical profiling of chlorophyll in North Atlantic Sea revealed phytoplankton populations to already start increasing in winter at very low rates, but which then accelerate during the spring months (Mignot, Ferrari and Claustre, 2018). The authors concluded that lateral variations in the environment influences bloom formation more so than biological factors (Mignot, Ferrari and Claustre, 2018).

A site of interest in the northeast Atlantic is the Faroe-Shetland Channel (FSC) as it is an area of high economic importance due to active oil exploration (Gallego *et al.*, 2018). It is recognised as subarctic region, and it has been identified as an important site for benthic marine life (Bett, 2001). For this reason, efforts in understanding the risk of oil pollution on marine life have been extensively explored in this area. The hydrodynamic and physicochemical characteristics of the FSC have also been investigated in order to model the potential trajectories of crude oil in the event of a major spill in this region (Main *et al.*, 2017; Gallego *et al.*, 2018). Biological characterisation to assess risk of crude oil pollution in FSC includes studies on the response of sediment bacterial community to oil enrichment and use of dispersant for mitigation of oil pollution (Suja, Summers and Gutierrez, 2017; Gontikaki *et al.*, 2018; Perez Calderon *et al.*, 2018).

However, no studies have shown the response of microorganisms in the subsurface seawater to crude oil pollution in addition to projected CO_2 enriched conditions. Microalgal-bacterial interaction heavily influences bloom formation (Amin *et al.*, 2015). As primary producers, phytoplankton supports the entire ecosystem by synthesising organic carbon, and the bacterial community associated to phytoplankton are responsible for nutrient cycling via the microbial loop and stimulates growth through signal communication (Amin *et al.*, 2015; Fuentes *et al.*, 2016; Dao *et al.*, 2018). Therefore, it is vital that we understand how this complex interaction between microbes will be affected by anthropogenically influenced environmental stressors such as ocean acidification and crude oil pollution. This study aims to understand the response of surface microbial community to crude oil pollution in current and future ocean conditions during spring phytoplankton bloom. The hypothesis is, if microalgal population thrives under high CO2 conditions, bacterial communities associated to microalgal populations will also thrive and be more capable of ecosystem functions such as removal of crude oil from seawater.

4.2. Methods

4.2.1. Field sampling

On 23rd May 2017, field sampling and on-board culturing of a mixed community of microbes on MV Scotia carried out in batch cultures with continuous aeration of ambient (400 ppm) or elevated CO_2 (750ppm) conditions. Subsurface seawater (5 m) samples from the Faroe-Shetland Channel (FSC) were collected using a rosette sampler coupled with CTD, as described in section 2.2.2 (Chapter 2). Salinity (35.23 o/00) and pH (7.4) were measured by CTD during sampling. Satellite observations detected chl *a* concentration between 0.272 to 0.281 mg/m^3 (Figure 4.1). Triplicates of 50 ml of homogenised spring phytoplankton community were preserved in Lugol's iodine and sent to SEPA for taxonomic identification and enumeration of the microalgal community. The phytoplankton community was expressed as mean percentage (%) of taxa per litre. Cultures were maintained for 8 days on-board MV Scotia before being transferred into laboratory conditions in Ashworth Building, University of Edinburgh for13 more days of culturing in microcosms treated with ambient (400 ppm) and elevated CO_2 (750 ppm). Throughout the entire experiment, light:dark cycle was maintained at 16:8 hours.

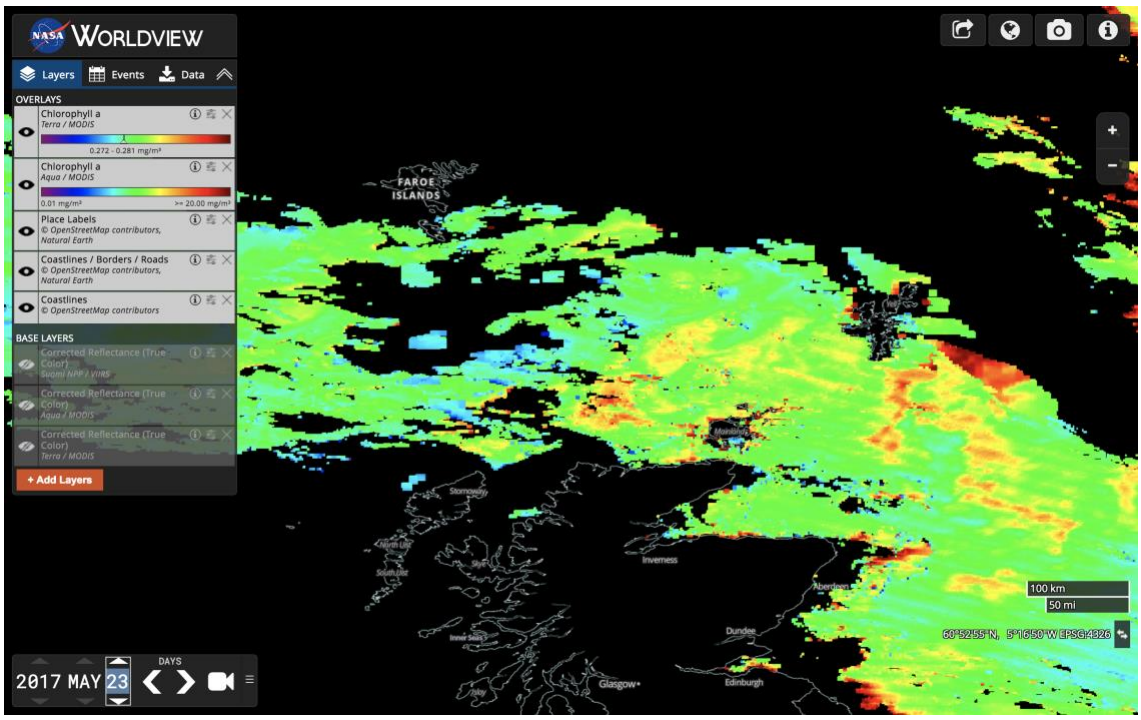


Figure 4.1. Satellite observation of chlorophyll *a* concentration (0.272 – 0.281 mg/m^3)in the FSC region during spring sampling (NASA Worldview, 2018).

4.2.2. Microcosm setup

Twenty litres of seawater were collected from FSC gently filtered through 120 µm sieve to exclude predatory large zooplankton (Suttle *et al.*, 1986) and homogenously mixed in a pre-autoclaved 10 L polypropylene carboy as the inoculum for microcosm experiments. Microcosm were filled with 400 ml of seawater collected from FSC treated with ambient/elevated CO_2 conditions and maintained under 4 conditions: 1) FSC surface seawater; biological control, 2) acid-killed/oil-enriched FSC surface seawater; hydrocarbon analysis control, 3) oil-enriched FSC surface seawater; biological analysis, and 4) oil-enriched FSC surface seawater. After 4 days of CO_2 acclimation in microcosms, the microbial community in the microcosms were exposed to 1% (v/v) Schiehallion crude oil.

4.2.3. Chlorophyll a abundance and pH readings

For chl *a* and pH analysis, microcosms were sampled on day 2, 4, 6, 9, 11, 13, 15, 18, and 21. The pH were measured immediately while the microalgal cells were pelleted by centrifugation, transferred to a clean tube and stored in acetone at -20 ^{0}C prior to fluorometric determination as detailed in section 2.3.1 (Chapter 2). Average readings were analysed using two-way ANOVA and Tukey's multiple comparison test to determine significance of treatments on chl *a* and pH.

4.2.4. Bacterial community analysis

For the purpose of comparing ambient and elevated CO_2 acclimated bacterial communities response to oil pollution, 5 ml of microcosm cultures were sampled at 4 timepoints: 1) baseline community of FSC prior to culturing (t0); 2) CO_2 acclimated community on day 4 (t1); 3) day 14 (t2) or 10 days after oil enrichment; 4) day 21 (t3), or 17 days after oil enrichment. Samples were filtered on polycarbonate membrane filters with a pore size of 0.22 μm and stored at -20 ^{0}C prior to potassium ethyl xanthogenate DNA extraction (Tillett and Neilan, 2000) as detailed in section 2.3.2 (Chapter 2). PCR-amplification of barcoded 16S rRNA, and purification of DNA in PCR using the GFX PCR DNA and Gel band purification kit (GE Healthcare) were done as described in section 2.3.2 (Chapter 2) prior to Illumina MiSeq sequencing at the Edinburgh Genomics Facility. Sequencing data were cleaned up and analysed using the mothur pipeline which includes constructing contiguous sequences, removal of homopolymers, alignment with SILVA database, and removal of chimera, and finally sequences were referenced to the RDP database, as described in section 2.3.2 (Chapter 2). Alpha diversity was analysed with one-way ANOVA of Shannon-diversity indices (H') in addition to visualisation using NMDS plots. Relative abundance of bacterial community were calculated using mean percentage of total bacterial community and comparing normalised (log2) ratio of median relative abundance of treatments. Heat tree diagram showing pairwise comparison of treatments were done using metacodeR R package (Foster, Sharpton and Grünwald, 2017). All statistical calculations were carried out on PRISM version 7 for Mac (GraphPad Software Inc).

4.2.5. Carbonate chemistry analysis

Seawater samples from FSC taken immediately from Niskin bottles and samples from microcosms at the end of experiment were transferred into 100 ml narrow necked glass bottles with minimal headspace before sealing the bottles with silicon free grease (Apiezon) as detailed in SOP 1 of Guide to Best Practices for Ocean CO_2 Measurements (Dickson, Sabine and Christian, 2007). Samples were stored in the dark at room temperature. Bottles containing samples were placed in water baths set at 25 ^{0}C prior to opening and analysis. Total dissolved inorganic carbon (DIC) was determined using acidification, gas stripping and infrared detection and described in Chapter 2. Total hydrogen ion were determined using Orion ROSS Ultra pH/ATC (ThermoFisher) glass electrode with a standard 2-amino-2-methyl-1,3-propanediol (Tris) buffer in synthetic

seawater as described in chapter 2. Calculation of carbonate chemistry parameters were done using co2calc (Robbins, Hansen and Meylan, 2016)

4.2.6. Hydrocarbon extraction and analysis

To analyse for biodegradation of the oil, total hydrocarbon extractions were performed at the termination (21 days) of these experiments with only microcosms that were left untouched (i.e. not sampled). Hydrocarbon from microcosms were extracted using dichloromethane as described in section 2.3.3 (Chapter 2). Hydrocarbon profiling was carried out by Dr. Faye Cruikshank from the School of Chemistry, University of Edinburgh, using atmospheric pressure photoionisation (APPI) coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) (Lemkau *et al.*, 2014; McKenna *et al.*, 2013).

4.3. Results

4.3.1. Characterisation of microplankton community

Surface seawater samples collected at 5 m deep in the FSC were taxonomically identified and enumerated (Figure 4.2). Triplicates of 50 ml samples fixed in Lugol's iodine showed surface seawater from the FSC taken during spring comprised of microflagellates, microalgae (coccolithophores, diatoms and dinoflagellate) and ciliates (Table 4.1; Figure 4.2). Microflagellates dominated the microbial community (62% ± 2; 15 x 10^4 cells/L), followed by diatoms (20% ± 3; 5 x 10^4 cells/L), coccolithophores (10% ± 2; 2.4 x 10^4 cells/L), dinoflagellates (8% ± 1; 1.9 x 10^4 cells/L), ciliates (<1%) and other microalgae groups (<1%; 1.7 x 10^3 cells/L; Figure 4.2). The coccolithophores identified were predominantly *E. huxleyi* (Table 4.1).

The spring microalgal community collected from surface of FSC described above (Figure 4.2) were acclimated in microcosms exposed to ambient (400 ppm) or elevated (750 ppm) atmospheric CO_2 treatments for 4 days. In no-oil control treatments, three exponential growth phases were observed throughout the 21-day duration of the experiment based on chl *a* measurement (Figure 4.3). After the initial death phase, which may be attributed to bottle effects i.e. non-specific consequences of the confinement in microcosms during the initiation of the experiment (Pernthaler and Amann, 2005), exponential growth phase began on day 2 in both ambient and elevated CO_2 treated microcosms.

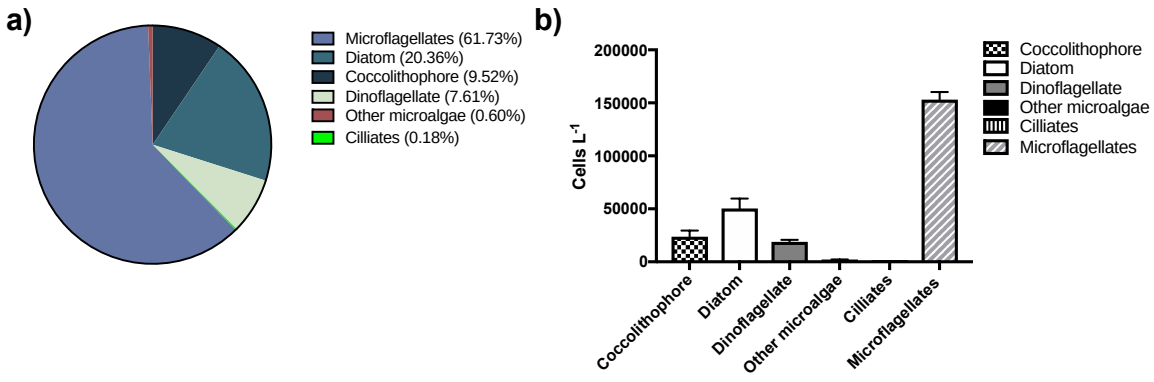


Figure 4.2. a) Composition of the microplankton community of spring surface (5 m) shown as mean percentages of total microalgal community collected from FSC, and b) cell counts (per litre) of the major microalgal lineages that were identified. Values are mean of cell concentration (n = 3) and error bars are standard error of mean.

Table 4.1. Summary of taxonomic analysis of microplankton community of FSC subsurface seawater sampled during spring. Values are mean % (n = 3) of taxon ± standard error of mean.

Lineage	Taxa	Mean % ± SEM
Microflagellate	Indeterminate microflagellate	57.45 ± 3.74
	Pyrenomonad	2.99 ± 0.79
Diatom	Indeterminate centric diatom	27.18 ± 0.06
	Dactyliosolen fragilissimus	0.40 ± 0.14
	Thalassiosira	0.24 ± 0.05
	Indeterminate raphiated pennate diatom	0.18 ± 0.06
	Pseudo-nitzschia	0.15 ± 0.04
	Dactyliosolen fragilissimus	0.40 ± 0.14
	Thalassiosira	0.24 ± 0.05
	Roperia tessellata	0.01 ± 0.01
	Coscinodiscus radiatus	0.01 ± 0.01
Coccolithophores	*Emiliania huxleyi*	9.28 ± 2.34
	Coccolithus pelagicus	0.22 ± 0.05
Dinoflagellate	*Gyrodinium flagellare*	5.13 ± 0.76
	Indeterminate unarmoured dinoflagellate	2.02 ± 0.18
	Prorocentrum cordatum	0.09 ± 0.05
	Indeterminate armoured dinoflagellate	0.06 ± 0.06
	Heterocapsa	0.06 ± 0.03
	Katodinium	0.03 ± 0.03
	Prorocentrum cordatum	0.09 ± 0.05
	Tripos fusus	0.06 ± 0.03
	Alexandrium	0.03 ± 0.03
	Dinoflagellate cyst	0.03 ± 0.01
	Tripos furca	0.02 ± 0.01
	Dinophysis acuminata	0.01 ± 0.00
	Protoperidinium subinerme	0.01 ± 0.00
	Tripos	0.01 ± 0.00
Other	*Raphidophyte*	0.30 ± 0.30
Cilliates	Indeterminate ciliated protist	0.10 ± 0.01
	Laboea strobila	0.06 ± 0.02
	Lohmaniella	0.01 ± 0.01

4.3.2. Microalgal response to ocean acidification and oil pollution

Microcosms filled with surface seawater from the FSC were acclimated to ambient (400 ppm) and elevated (750 ppm) CO_2 concentration. Microalgal response observed by chl *a* concentration shows significant differences after 4 days of CO_2 acclimation (Figure 4.3). Chl *a* concentration of ambient/without oil and elevated CO_2 prior to oil-enrichment were significantly different ($p = 0.0103$) on day 4. Chl *a* concentration in ambient/oil-enriched and elevated CO_2/oil-enriched treated microcosms were not significantly different on day 6, however significantly higher chl *a* was detected in elevated CO_2/without oil microcosms compared to ambient/without oil ($p = 0.0063$) treatments, and elevated CO_2/oil-enriched ($p < 0.0001$) treatments.

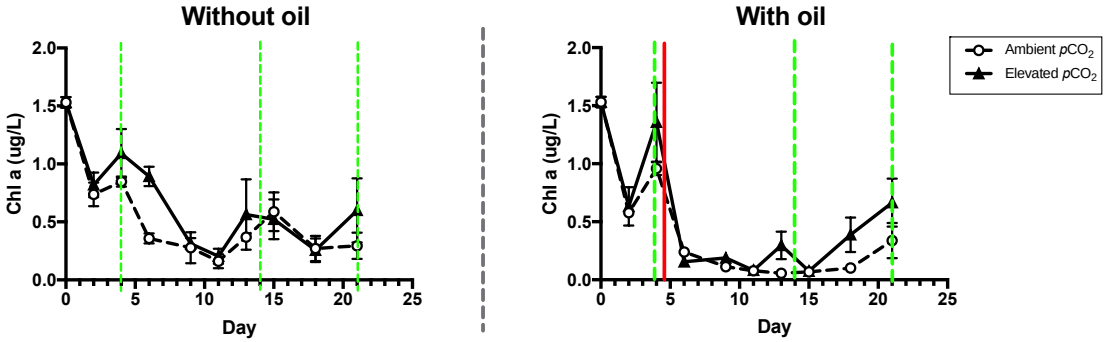


Figure 4.3. Growth response of microalgae in microcosms treated with ambient (open circles) and elevated CO_2 (closed triangles) in no-oil controls (left) and with oil enrichment (right). Oil was added on day 4 (indicated by red lines), while samples for 16S rRNA sequencing were taken at three timepoints (indicated by green lines): t1 (day 4 prior to oil enrichment), t2 (day 14), and t3 (day 21).

No significant differences were detected between all treatments as chl *a* concentration were very low on day 9 and day 11 (Figure 4.3). Microalgal community in microcosms without oil recovered from death phase and chl *a* concentration of elevated CO_2/without oil treated microcosms were significantly higher than ambient/oil-enriched microcosms ($p = 0.0109$) on day 13.

Recovery from death phase and into exponential growth phase continued for both ambient and elevated CO_2 treated microcosms without oil enrichment on day 15 while chl *a* concentration in oil-enriched microcosms were almost undetected (Figure 4.3). Chl *a* concentration increased in microcosm enriched with oil but decreased in microcosms without oil on day 18. Chl *a* concentration continued to increase in microcosms enriched with oil, and in elevated CO_2/without oil but not in ambient/without oil. However, no

significant differences of chl *a* concentration were detected between all treatments on day 18 and day 21.

4.3.3. Bacterial community response

Bacterial community sampled directly from FSC were baseline (t0) samples prior to acclimation in microcosms to ambient or elevated CO_2 and exposing microcosms with or without oil-enrichment. The Shannon diversity index (H') was used to assess abundance and evenness of taxa of bacterial communities acclimated to ambient (400ppm) and elevated (750 ppm) CO_2 treated microcosms with/without oil-enrichment (Figure 4.4). Oil-enriched/elevated CO_2 treated microcosms show reduced H' at t1 and t2 compared to t0, while average of H' of oil-enriched/ambient treated microcosms were higher at t1, t2, and t3 (Figure 4.4). When compared to the baseline community of sampled subsurface seawater collected from FSC, no significant differences of H' were observed ($p > 0.05$; Figure 4.4). H' of elevated CO_2/without oil were significantly higher than ambient/without oil at t1 ($p = 0.0436$), in addition to significantly higher H' of elevated CO_2/without oil compared to H' of elevated CO_2/oil-enriched microcosm at t2 ($p=0.0066$). No significant differences were detected at t3 ($p > 0.05$).

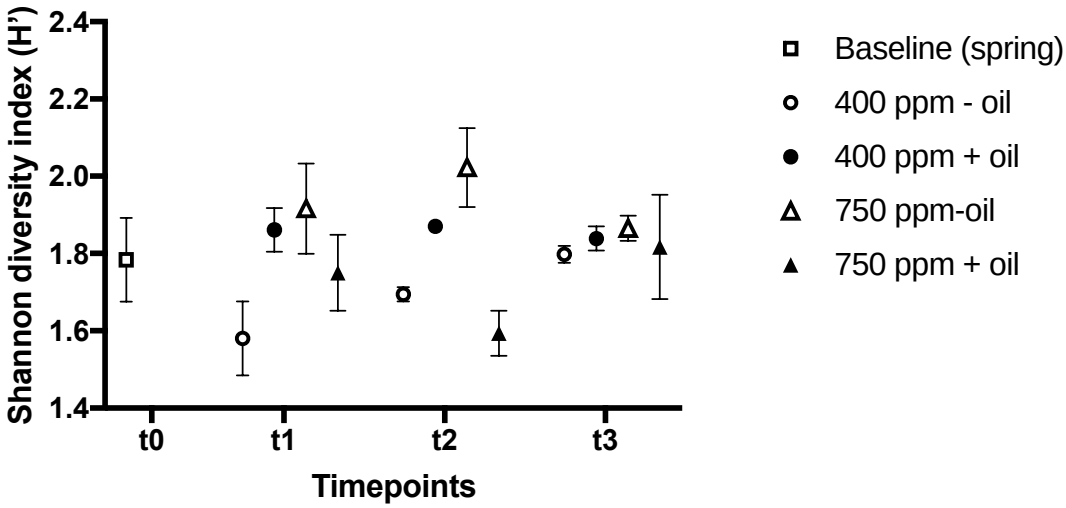


Figure 4.4. Shannon diversity index (H') of the bacterial diversity between treatments over time. Values show are mean of triplicates (n = 3) at each timepoint with error bars showing SEM.

Cluster analysis of baseline bacterial community were compared with bacterial community in microcosms using Bray Curtis similarity matrix to identify dissimilarity of bacterial community in different treatments or timepoints (Figure 4.5). Triplicates of the baseline samples were 40% similar (Figure 4.5). All of the bacterial community from

microcosms were at least 80% dissimilar from baseline (Figure 4.5). Most treatments were 50% dissimilar from baseline, except for one replicate from each timepoint of microcosm treated with elevated CO_2 without oil enrichment. These samples were 30% dissimilar from each other even though samples were of different timepoints. No distinct separation between CO_2 treatment, or oil-enrichment were seen between samples at t1, t2 and t3 (Figure 4.5). ANOSIM pairwise test identified low separation between elevated CO_2 and oil treatments with elevated CO_2 without oil (R-statistic 0.278, p = 0.04) and with ambient CO_2 with oil (R-statistics 0.463, p = 0.01). No other treatments were significantly different from each other (p > 0.05). ANOSIM pairwise comparison of timepoints shows no significant differences between each timepoint.

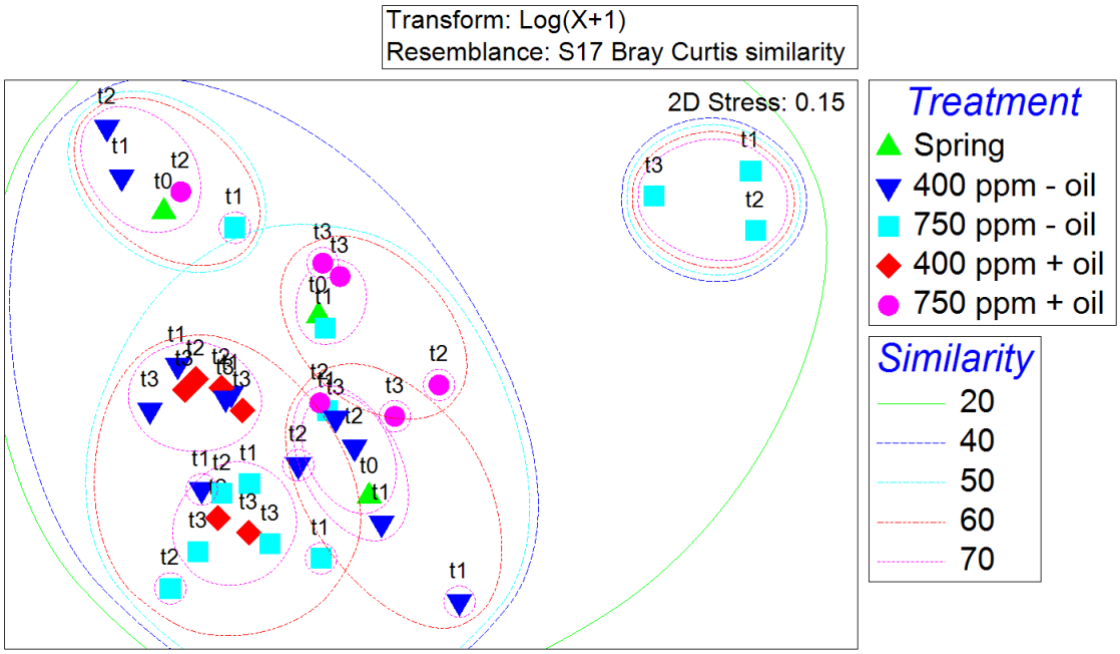


Figure 4.5. NMDS plots based on Bray Curtis similarity index of the bacterial community associated with the spring microalgal community in microcosms treated with ambient/elevated CO_2, and with/without oil enrichment at four timepoints (t0 representing the baseline prior to culturing; t1representing day 4 prior to oil enrichment; t2 representing day 14; and t3 representing day 21). Treatments are represented by coloured shapes shown in legend where 'Spring' is the baseline sample at t0; '400 ppm' or '750 ppm' is the CO_2 concentration (ambient or elevated respectively), and '+ oil' or '- oil' is the presence or absence of oil respectively. Clusters based on log (X + 1) Bray-Curtis similarity index (%) are indicated by coloured ellipses.

Following collective bacterial community analysis, taxon specific comparison in each treatment was done by comparing relative abundance of (log2) median in heat tree diagrams (Figure 4.6 and Figure 4.7) in addition to calculating mean percentage of

bacterial taxa in total bacterial community (Table 4.2). Larger tree (left) shows all taxa present in this study. Species richness in bacterial community subjected to only CO_2 stress were higher (72 OTU's) compared to bacterial community subjected to oil enrichment (67 OTU's). Only significantly different log2 median ratios of paired treatments determined by Wilcox rank-sum test and Benjamini Hochberg (FDR) correction for multiple comparisons were shown corresponding to intensity of green or brown branches in the smaller heat trees (Figure4.6 and Figure 4.7). Heat tree allows for a more detailed comparison of OTU's at all levels if compared to stacked bar charts that are limited by colours displayed on a phylogenetic tree (Foster, Sharpton and Grünwald, 2017).

Baseline microbial community collected in spring

High relative abundance of *Colwellia* (16% ± 9), unclassified *Rhodobacteraceae* (16% ± 7), unclassified *Gammaproteobacteria* (11% ± 3), unclassified bacteria (7% ± 3), unclassified *Candidatus* Pelagibacter (6% ± 3), *Psychrobacter* (5% ± 5), unclassified *Alteromonadaceae* (5% ± 3) and unclassified *Flavobacteriaceae.* (5% ± 2) were observed in baseline sample of seawater collected from surface of FSC (Table 4.2). After four days in microcosm conditions (t1), relative abundance of *Pseudoalteromonas*, unclassified *Alteromonadale*s, unclassified *Alteromonadaceae*, unclassified *Betaproteobacteria*, and unclassified *Flavobacteriaceae* were significantly reduced in both CO_2 treatments when compared to baseline bacterial community (t0; Figure 4.6).

Enrichment of *Polaribacter* (1%), and unclassified bacteria (1 – 9%) were observed after acclimation to ambient CO_2 treatments. Only unclassified *Alphaproteobacteria* decreased in relative abundance when treated with ambient CO_2 (1 – 3%). High relative abundance of taxa (>5%) after 4 days of ambient CO_2 treated microcosm conditions includes *Colwellia* (27% ± 8), unclassified *Rhodobacteraceae* (12% ± 2), unclassified *Gammaproteobacteria* (12% ± 2), unclassified bacteria (9% ± 4), and unclassified *Flavobacteriaceae* (5% ± 2).

Bacterial community after 4 days of elevated CO_2 treatment

In comparison, bacterial community in elevated CO_2 conditions on day 4 were dominated (>5%) by unclassified *Gammaproteobacteria* (15% ± 3), *Colwellia* (13% ± 4), unclassified *Rhodobacteraceae* (12% ± 2), and unclassified bacteria (5% ± 2). Relative abundance of *Colwellia* reduced by 2 – 24%, unclassified bacteria decreased by 1 – 10%, *Polaribacter* by 1 – 2%, and unclassified *Flavobacteriaceae* by 1 – 4% when

treated with elevated CO_2 conditions (Table 4.2). Only relative abundance of unclassified *Alphaproteobacteria* increased (1 – 2%) from higher CO_2 conditions. Elevated CO_2 conditions were found to enrich the order *Oceanospirillales* and *Pseudomonadales* (Figure 4.6).

Bacterial community after 14 days of elevated CO_2 treatment

Higher relative abundance of unclassified *Halomonadaceae* (1 – 8%), *Aliivibrio* (1 – 7%), *Pseudomonas* (1 - 2%), unclassified *Vibrionaceae* (1 – 2 %), *Sulfitobacter* (1%), and unclassified *Colwelliaceae* (1%) in elevated CO_2 treated microcosms were observed compared to ambient treated microcosms (Figure 4.6 and Table 4.2). Negatively affected taxa under elevated CO_2 at t2 shows decrease in relative abundance of *Colwellia* (3 – 15%), unclassified *Alteromonadaceae* (2 – 8%), and unclassified *Flavobacteriaceae* (1 – 6%) (Figure 4.6 and Table 4.2). *Alteromonadales*, unclassified *Candidatus* Pelagibacter, and unclassified *Alphaproteobacteria* were also negatively affected, but at a smaller extent where 1 -3% decrease in relative abundance were observed for each taxon.

Dominating taxa under elevated CO_2 conditions at t2 were unclassified *Gammaproteobacteria* (22% ± 5), unclassified *Rhodobacteraceae* (10% ± 3), *Colwellia* (9% ± 4) unclassified *Halomonadaceae* (5% ± 3) and *Aliivibrio* (5% ± 2) while dominating taxa under ambient conditions at t2 were *Colwellia* (18% ± 2), unclassified *Rhodobacteraceae* (12% ± 0), unclassified *Gammaproteobacteria* (11% ± 2), unclassified bacteria, (6% ± 1), unclassified *Alteromonadaceae* (6% ± 3), unclassified *Flavobacteriaceae* (5% ± 2), and *Psychrobacte*r (5 % ± 4; Table 4.2).

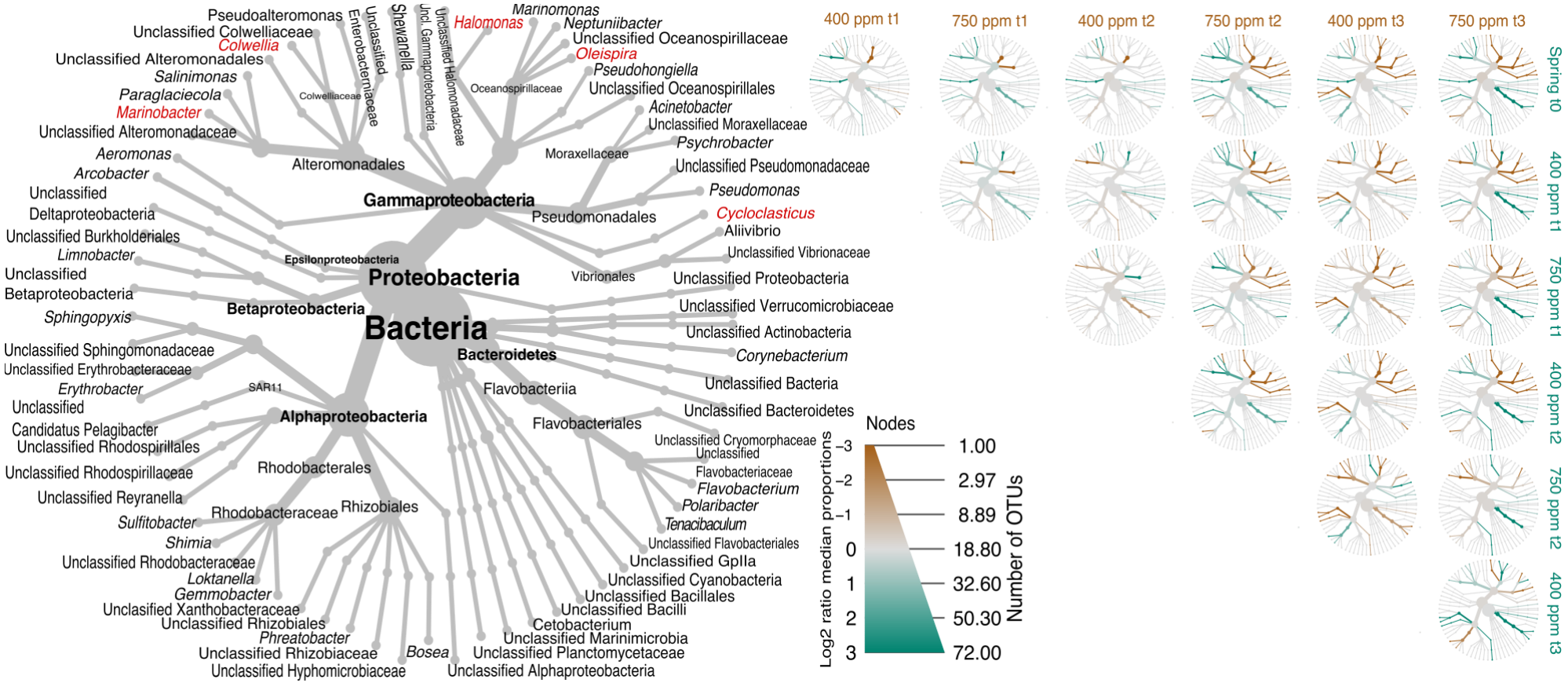


Figure 4.6. Heat tree showing pairwise comparison of the microbial community exposed to ambient and elevated CO_2 treatments at different timepoints. The larger tree on left displays all the OTUs identified, including related taxa of cultivated species with known hydrocarbonoclastic potential (shown in red). The diameter of nodes on the larger tree corresponds to qualitative number of OTU's in the respective taxa. Smaller trees represent significantly enriched taxa between paired treatments; ambient (400 ppm) or elevated CO_2 (750 ppm) at different timepoints corresponding to horizontal (brown) and vertical (green) axis. Sampling timepoints were t0 (the baseline in-situ community); t1 at day 4; t2 at day 14; t3 at day 21. Intensity of colour in smaller trees (brown or green) corresponds to difference in ratio of relative abundance of taxa in paired treatments.

Bacterial community after 22 days of elevated CO_2 treatment

Elevated CO_2 treated microcosms at t3 increased relative abundance of unclassified *Rhodobacteraceae* (1 - 11%), unclassified *Halomonadaceae* (4 – 8%), Acinetobacter (1 – 3%), and *Sulfitobacter* (1 -3%; Table 4.2). Large decrease in relative abundance of *Colwellia* (2 - 14%) was seen again at t3 of elevated CO_2 treated microcosm. Besides Colwellia, only smaller decrease of relative abundance were seen when microcosms were treated with elevated CO_2. For example, relative abundance of unclassified bacteria were 2 – 4% less, unclassified *Colwelliaceae* decreased by 1 - 6%, *Marinomonas* and unclassified *Flavobacteriaceae decreased* by 1 – 5%, unclassified *Alphaproteobacteria* by 1 – 4%, *Polaribacter and* unclassified *Oceanospirillaceae* by 1 - 3%, *Erythrobacter*, unclassified *Alteromonadaceae* and unclassified *Erythrobacteraceae* by 1% (Table 4.2). At t3, dominating taxa under elevated CO_2 conditions without oil enrichment were unclassified *Gammaproteobacteria* (17%% ± 6), unclassified *Rhodobacteraceae* (13% ± 1), *Colwellia* (11% ± 5), unclassified *Rhizobiales* (10 % ± 10) and unclasified *Planctomycetaceae* (5% ± 4). Meanwhile, ambient treated microcosms were dominated by *Colwellia* (24% ± 6), *u*nclassified *Gammaproteobacteria* (19% ± 6), unclassified *Rhodobacteraceae* (8% ± 5), and unclassified *Colwelliaceae* (5% ± 2; Table 4.2).

Bacterial community response after 9 days of oil-enrichment in elevated CO_2 condition

Despite *Colwellia* being negatively impacted by elevated CO_2 treatments, relative abundance of *Colwellia* increased by 1 - 33% when elevated CO_2 treated microcosm were exposed to crude oil (Table 4.2). Other bacterial taxa that were enriched when oil enrichment was treated with elevated CO_2 conditions were unclassified *GpIIa* (1 – 3%), and *Oleispira* (1%) (Figure 4.7; Table 4.2). Oil enrichment reduced relative abundance of unclassified *Gammaproteobacteria (1 - 9%), Aliivibrio* (3 – 5%), unclassified *Flavobacteriaceae (2 – 4%),* and unclassified *Alphaproteobacteria (1 – 3%)* at t2. Relative abundance of *Marinomonas*, *Polaribacter*, unclassified *Vibrionaceae*, and unclassified *Alphaproteobacteria* were only reduced by 1%.

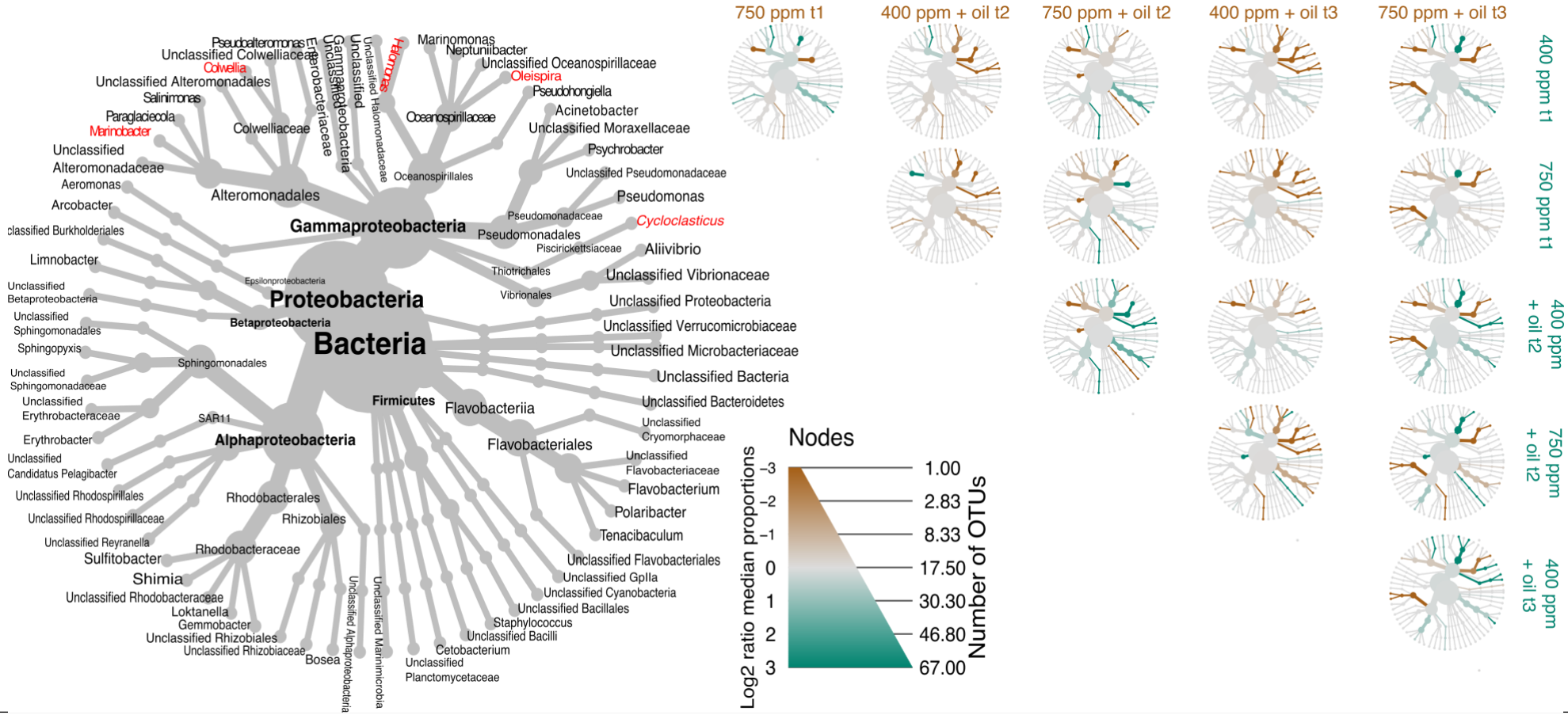


Figure 4.7. Heat tree showing pairwise comparison of the microbial community exposed to crude oil enrichment in ambient or elevated CO_2 treatments at different timepoints. The larger tree on left displays all the OTUs identified, including related taxa of cultivated species with known hydrocarbonoclastic potential (shown in red). The diameter of nodes on the larger tree corresponds to qualitative number of OTU's in the respective taxa. Smaller trees represent significantly enriched taxa between paired treatments; ambient (400 ppm) or elevated CO_2 (750 ppm) at different timepoints corresponding to horizontal (brown) and vertical (green) axis. Sampling timepoints were t0 (the baseline in-situ community); t1 at day 4; t2 at day 14; t3 at day 21 Intensity of colour in smaller trees (brown or green) corresponds to difference in ratio of relative abundance of taxa in paired treatments.

Despite the shift in mean percentage of taxa, the dominating taxa in the bacterial community in elevated CO_2/oil-enriched treated microcosms at t2 were similar to ambient/oil-enriched bacterial community. *Colwellia* (28 % ± 14), unclassified *Rhodobacteraceae* (18% ± 10), unclassified *Gammaproteobacteria* (10% ± 1), and unclassified bacteria (9% ± 6) were dominant in elevated conditions at t2, and ambient/oil-enriched bacterial community at t2 were also dominated by *Colwellia* (19% ± 1), unclassified *Gammaproteobacteria* (19% ± 1), unclassified *Rhodobacteraceae* (14% ± 1), and unclassified bacteria (5% ± 2).

Bacterial community response after 17 days of oil-enrichment in elevated CO_2 condition

After 16 days of oil exposure, increase in relative abundance of *Colwellia* (1 - 19%), unclassified *Alteromonadaceae* (2 – 8%), *Psychrobacter* (2 – 7%), *Sphingopyxis* (1%), and unclassified *Sphingomonadaceae* (1 – 2%), were significantly enriched under elevated CO_2 conditions (Figure 4.7; Table 4.2). Meanwhile, decrease in relative abundance of unclassified *Gammaproteobacteria* (6 - 14%), *Aliivibrio* (3 – 7%), *Marinomonas* (1 – 5%), unclassified *Rhodobacteraceae* (1 - 5%), unclassified *Alphaproteobacteria* (2 – 3%), unclassified *Vibrionaceae* (1- 3%), unclassified *Colwelliacea* (1 – 2%), unclassified *Oceanospirillaceae* (1 – 2%), *Pseudomonas* (1%), and *Polaribacter* (1%) were significantly reduced under oil-enriched/elevated CO_2 conditions compared to oil-enriched/ambient conditions at t3 (Figure 4.7; Table 4.2).

Colwellia (30% ± 5), unclassified *Rhodobacteraceae* (9% ± 1), unclassified *Gammaproteobacteria* (8% ± 1), unclassified bacteria (7% ± 1), *Psychrobacter* (6% ± 3), and unclassified *Alteromonadaceae* (6% ± 3) dominated elevated/oil-enriched CO_2 treated microcosms. In comparison, highly abundant taxa at the end of experiment when microcosms were oil-enriched under ambient CO_2 conditions were *Colwellia* (21% ± 5), unclassified *Gammaproteobacteria* (18% ± 3), unclassified *Rhodobacteraceae* (12% ± 1), *Aliivibrio* (5% ± 2), and unclassified bacteria (5% ± 2) (Table 4.2).

Table 4.2. Average relative abundance (%) ± standard error of spring bacterial OTU count between ambient (400 ppm)/elevated CO_2 (750 ppm) and enrichment/absence of crude oil (+/-) across timepoints (t0; baseline, t1; day 5, t2; day 15, and t3; day 22). Relative abundance (%) were rounded to nearest number and OTU's were grouped into respective phyla.

Timepoint	**t0**	**t1**		**t2**				**t3**			
CO_2 treatment		**400 ppm**	**750 ppm**	**400 ppm**		**750 ppm**		**400 ppm**		**750 ppm**	
Oil enrichment											
Oil enrichment		**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
No. of OTU's	**491 ± 114**	**494 ± 88**	**415 ± 41**	**751 ± 299**	**691 ± 40**	**404 ± 103**	**525 ± 75**	**643 ± 144**	**640 ± 130**	**586 ± 93**	**467 ± 80**
Unclassified bacteria	7 ± 3	9 ± 4	5 ± 2	6 ± 1	5 ± 2	3 ± 1	9 ± 6	4 ± 1	5 ± 2	1 ± 0	7 ± 1
Bacteroidetes (Phyla)											
Unclassified *Bacteroidetes*	1 ± 0	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1± <1	1 ± <1	<1 ± <1	<1 ± <1	<1± <1	1 ± <1
Unclassified *Cryomorphaceae*	1 ± 0	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1
Unclassified *Flavobacteriaceae*	5 ± 2	5 ± 2	4 ± 1	5 ± 2	3 ± 1	2 ± 1	1 ± <1	4 ± 2	3 ± 1	1 ± 0	3 ± 1
Flavobacterium	0 ± 0	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	0 ± 0	0 ± 0	<1 ± <1	<1 ± <1
Polaribacter	2 ± 2	1 ± 0	2 ± 1	3 ± 2	2 ± 0	<1 ± <1	<1 ± <1	2 ± 1	1 ± 1	<1 ± <1	1 ± <1
Tenacibaculum	1 ± 1	1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1
Cyanobacteria (Phyla)											
Unclassified *GpIIa*	1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	2 ± 1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1
Unclassified *Cyanobacteria*	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1
Firmicutes (Phyla)											
Unclassified *Bacillales*	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	0 ± 0	1 ± 1
Staphylococcus	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1

Continued Table 4.2 part 1/4

Timepoint	t0	t1		t2				t3			
CO_2 treatment		400 ppm	750 ppm	400 ppm		750 ppm		400 ppm		750 ppm	
Oil enrichment / OTU	-	-	-	-	+	-	+	-	+	-	+
Fusobacteria (Phyla)											
Cetobacterium	<1 ± <1	<1 ± <1	1 ± 1	1 ± <1	<1 ± <1	3 ± 3	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1
Planctomycete (Phyla)											
Unclassified *Planctomycetaceae*	<1 ± <1	<1 ± <1	2 ± 2	1 ± 1	<1 ± <1	3 ± 3	<1 ± <1	<1 ± <1	<1 ± <1	5 ± 4	<1 ± <1
Proteobacteria (Phyla)											
Unclassified *Proteobacteria*	3 ± 1	3 ± 1	3 ± 1	3 ± <1	3 ± 1	3 ± 1	4 ± 1	3 ± <1	2 ± <1	2 ± 1	2 ± 1
Alphaproteobacteria (Class)											
Unclassified *Alphaproteobacteria*	3 ± 2	2 ± <1	3 ± 1	3 ± 1	2 ± 1	2 ± 1	2 ± 1	3 ± <1	3 ± 1	4 ± 3	2 ± 1
Bosea	<1 ± <1	0 ± 0	<1 ± <1	<1 ± <1	0 ± 0	<1 ± <1	<1 ± <1	0 ± 0	<1 ± <1	1 ± 1	<1 ± <1
Unclassified *Hyphomicrobiaceae*	0 ± 0	0 ± 0	<1 ± <1	<1 ± <1	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	<1 ± <1	<1 ± <1
Unclassified *Rhizobiaceae*	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	0 ± 0	1 ± 1	0 ± 0	<1 ± <1	0 ± 0	1 ± 1	<1 ± <1
Unclassified *Rhizobiales*	0 ± 0	0 ± 0	3 ± 3	1 ± 1	<1 ± <1	4 ± 4	<1 ± <1	<1 ± <1	0 ± 0	10 ± 10	<1 ± <1
Gemmobacter	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	2 ± 1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1
Loktanella	1 ± 0	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1

Continued Table 4.2 part 2/4

Timepoint	**t0**	**t1**		**t2**				**t3**			
CO_2 Treatment		**400 ppm**	**750 ppm**	**400 ppm**		**750 ppm**		**400 ppm**		**750 ppm**	
Oil enrichment / **OTU**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
Unclassified *Rhodobacteraceae*	16 ± 7	12 ± 2	12 ± 2	12 ± 0	14 ± 1	10 ± 3	18 ± 10	8 ± 5	12 ± 1	13 ± 1	9 ± 1
S*ulfitobacter*	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 1	1 ± 1	1 ± 0	1 ± 1	1 ± 0	2 ± 1	1 ± 0
Unclassified *Reyranella*	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	1 ± 1	0 ± 0
Unclassified *Rhodospirillaceae*	1 ± 1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	1 ± <1	<1 ± <1	<1 ± <1	1 ± <1
Unclassified *Cand. Pelagibacter*	6 ± 3	4 ± 2	4 ± 2	3 ± 1	3 ± 1	1 ± <1	3 ± 1	3 ± 2	2 ± 1	<1 ± <1	3 ± 1
Erythrobacter	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	1 ± 1
Unclassified *Erythrobacteraceae*	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	1 ± 1
Unclassified *Sphingomonadaceae*	<1 ± <1	0 ± 0	<1 ± <1	1 ± 1	0 ± 0	<1 ± <1	1 ± 1	0 ± 0	0 ± 0	<1 ± <1	1 ± 1
Sphingopyxis	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	0 ± 0	1 ± 0
Unclassified *Sphingomonadales*	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	0 ± 0	<1 ± <1	0 ± 0	<1 ± <1	0 ± 0	1 ± 1
Betaproteobacteria (Class)											
Unclassified *Betaproteobacteria*	1 ± <1	1 ± <1	1 ± 1	1 ± <1	<1 ± <1	2 ± 1	1 ± <1	<1 ± <1	1 ± <1	1 ± <1	1 ± <1
Limnobacter	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	0 ± 0	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1

Continued Table 4.2 part 3/4

Timepoint	**t0**	**t1**		**t2**				**t3**			
CO_2 Treatment		**400 ppm**	**750 ppm**	**400 ppm**		**750 ppm**		**400 ppm**		**750 ppm**	
Oil enrichment											
OTU	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
Unclassified *Burkholderiales*	<1 ± <1	0 ± 0	<1 ± <1	1 ± 1	0 ± 0	0 ± 0	1 ± 1	0 ± 0	<1 ± <1	<1 ± <1	<1 ± <1
Gammaproteobateria (Class)											
Aeromonas	0 ± 0	<1 ± <1	1 ± <1	<1 ± <1	0 ± 0	1 ± 1	0 ± 0	<1 ± <1	0 ± 0	<1 ± <1	0 ± 0
Unclassified Alteromonadaceae	5 ± 3	1 ± <1	3 ± 2	6 ± 3	<1 ± <1	1 ± <1	1 ± 1	1 ± <1	1 ± <1	1 ± <1	6 ± 3
Marinobacter	<1 ± <1	1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1
Unclassified *Alteromonadales*	4 ± 2	2 ± 1	2 ± 1	3 ± 1	1 ± <1	1 ± <1	1 ± 1	2 ± <1	2 ± 1	2 ± 1	4 ± 2
Colwellia	16 ± 9	27 ± 8	13 ± 4	18 ± 2	19 ± 1	9 ± 4	28 ± 14	24 ± 6	21 ± 5	11 ± 5	30 ± 5
Unclassfied *Colwelliaceae*	1 ± 1	3 ± 1	3 ± 1	2 ± <1	3 ± 1	2 ± 1	2 ± 1	5 ± 2	4 ± 1	2 ± 1	2 ± 1
Thalassotalea	0 ± 0	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1
Pseudoalteromonas	1 ± 1	1 ± <1	1 ± <1	1 ± <1	1 ± <1	1 ± <1	1 ± <1	<1 ± <1	1 ± <1	1 ± 1	1 ± <1
Shewanella	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1
Unclassified *Enterobacteriaceae*	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	3 ± 2	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1
Unclassified *Gammaproteobacteria*	11 ± 3	12 ± 2	15 ± 3	11 ± 2	19 ± 1	22 ± 5	10 ± 1	19 ± 6	18 ± 3	17 ± 6	8 ± 1
Unclassified *Halomonadaceae*	<1 ± <1	1 ± 1	2 ± 2	1 ± 1	<1 ± <1	5 ± 3	<1 ± <1	<1 ± <1	1 ± 1	4 ± 4	<1 ± <1
Halomonas	<1 ± <1	<1 ± <1	1 ± 0	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	1 ± 1	1 ± 1	<1 ± <1

Continued Table 4.2 part 4/4

Timepoint	**t0**	**t1**		**t2**				**t3**			
CO_2 Treatment		**400 ppm**	**750 ppm**	**400 ppm**		**750 ppm**		**400 ppm**		**750 ppm**	
Oil enrichment											
OTU		**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
Marinomonas	1 ± <1	1 ± <1	1 ± <1	1 ± <1	1 ± 1	1 ± <1	<1 ± <1	4 ± 2	3 ± 2	2 ± 1	<1 ± <1
Neptuniibacter	0 ± 0	1 ± 1	<1 ± <1	<1 ± <1	2 ± 1	0 ± 0	<1 ± <1	1 ± 1	1 ± 1	<1 ± <1	<1 ± <1
Unclassified *Oceanospirillaceae*	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	2 ± 2	<1 ± <1	<1 ± <1	2 ± 1	1 ± 1	<1 ± <1	<1 ± <1
Oleispira	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1
Unclassified *Oceanospirillales*	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1
Acinetobacter	<1 ± <1	1 ± 1	1 ± <1	<1 ± <1	1 ± 1	1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	2 ± 1	<1 ± <1
Psychrobacter	5 ± 5	1 ± <1	3 ± 2	5 ± 4	1 ± < 1	1 ± <1	4 ± 4	1 ± <1	1 ± <1	1 ± <1	6 ± 3
Unclassified *Pseudomonadaceae*	0 ± 0	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	0 ± 0	0 ± 0	<1 ± <1	<1 ± <1	0 ± 0
Pseudomonas	<1 ± <1	1 ± <1	1 ± <1	1 ± <1	1 ± <1	2 ± 1	<1 ± <1	<1 ± <1	1 ± <1	1 ± <1	<1 ± <1
Cycloclasticus	0 ± 0	<1 ± <1	<1 ± <1	<1 ± <1	2 ± 2	1 ± 1	0 ± 0	<1 ± <1	<1 ± <1	2 ± 2	<1 ± <1
Aliivibrio	<1 ± <1	1 ± 0	3 ± 2	1 ± 1	4 ± 1	5 ± 2	0 ± 0	3 ± 1	5 ± 2	4 ± 2	0 ± 0
Unclassified *Vibrionaceae*	<1 ± <1	1 ± <1	1 ± 1	1 ± <1	2 ± <1	2 ± 1	<1 ± <1	2 ± 1	2 ± 1	2 ± 1	<1 ± <1
Epsilonproteobacteria (Class)											
Arcobacter	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1

4.3.3. Carbonate chemistry of seawater in microcosms

Target levels of elevated pCO_2 (750 ppm) were higher than achieved levels of pCO_2 (730 ppm) due to the method of real-time mixing of ambient air (405 ppm of CO_2) were mixed with compressed CO_2 using mass flow controller (Figure 4. 8). The elevated pCO_2 slope was not significantly different from ambient pCO_2 slope ($p = 0.973$) while the differences between the elevations of the y-intercept are significantly different ($p < 0.0001$) suggesting CO_2 enrichment were consistent throughout the experiment and variability of elevated CO_2 daily average was not violated by CO_2 enrichment (Figure 4.8).

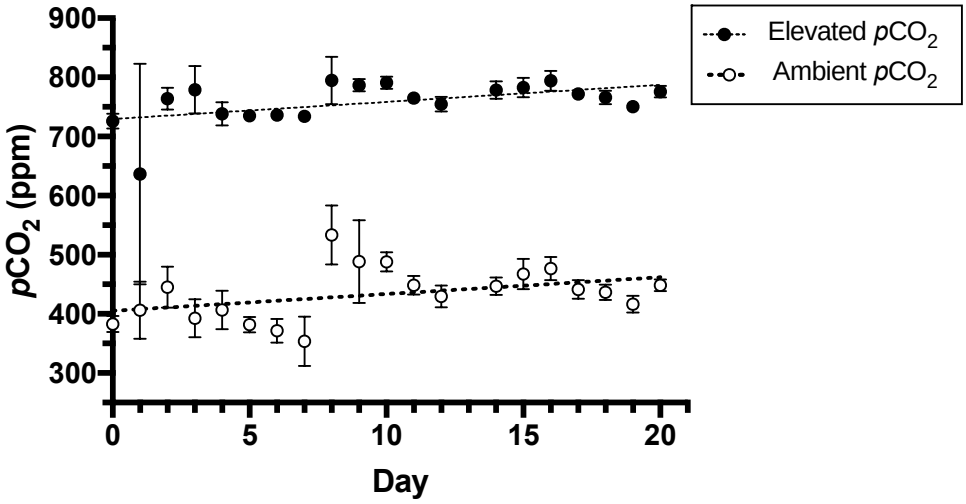


Figure 4.8. Daily average of CO_2 concentration (ppm) supplied to microcosms exposed to ambient and elevated pCO_2 conditions with linear regression (dotted lines) showing achieved level of ambient CO_2 (405 ± 18 ppm) and elevated pCO_2 (730 ± 13 ppm) concentration exposed to microcosms. Error bars shows standard deviation of pCO_2 per day.

After 2 days of culturing in microcosms, pH of microcosm increased by 0.4 units in all treatments except for ambient treated microcosms prior to oil enrichment, where pH increased only by 0.2 units (Figure 4.9). A difference of 0.2 pH units were significantly different ($p = 0.0007$) between ambient (400 ppm) and elevated (750 ppm) treated microcosms on day 4 prior to oil enrichment. After oil enrichment (day 6), pH of elevated CO_2/oil-enriched treated microcosms were significantly lower ($p = 0.0219$) than ambient/oil-enriched treated microcosms. Microcosms without oil were significantly different between CO_2 treatments ($p = 0.0088$) and significant differences were also seen between CO_2 treatments in oil-enriched microcosms ($p = 0.0219$) on day 6. No significant

differences were observed between oil-enriched and without oil enrichment in ambient or elevated CO_2 conditions ($p > 0.05$) on day 6.

Oil-enriched microcosms had significantly higher pH levels on day 9 compared to the respective non-oil controls; ambient ($p = 0.01219$) and elevated CO_2 ($p = 0.0412$). No significant differences were observed on day 11 to day 21 when oil-enriched microcosms were compared to microcosms treated with their respective CO_2 concentration without oil enrichment (Figure 4.3). pH of ambient/oil-enriched microcosms were significantly higher compared to microcosms treated with elevated CO_2/oil-enriched on day 9 ($p = 0.0042$), day 11 ($p < 0.0001$), day 13 ($p = 0.0007$), day 15 ($p = 0.0001$), and day 18 ($p = 0.0012$), but not significantly different on day 21 ($p>0.05$).

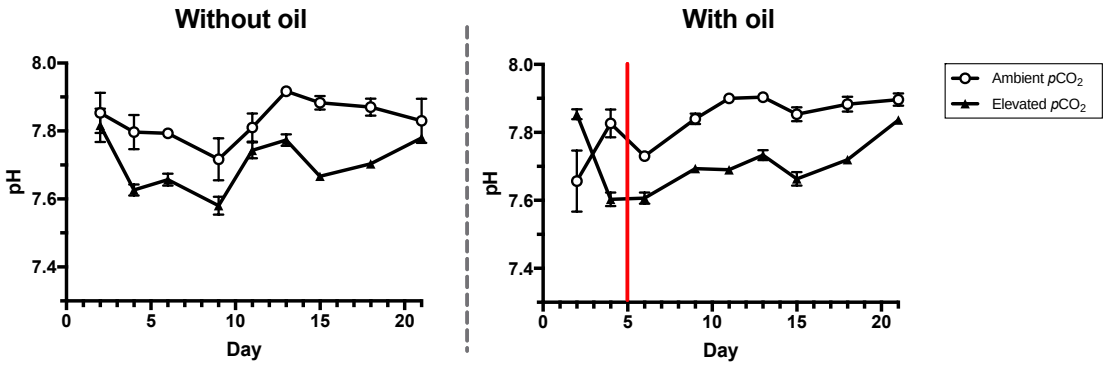


Figure 4.9. pH of seawater in microcosms treated with ambient (open circles) and elevated CO_2 (closed triangles) in no-oil controls (left) and with oil enrichment (right). Oil was added on day 4 (indicated by red lines).

Total dissolved inorganic carbon (C_T) analysis of seawater in microcosms at the end of experiment (day 21) were compared to baseline of spring seawater sampled from FSC using 2-way ANOVA and Tukey's multiple comparison test (Appendix A). C_T was significantly lower in baseline samples compared to elevated CO_2/oil-enriched treated microcosms ($p = 0.0007$), and elevated CO_2/without crude oil ($p = 0.0016$) as expected (Appendix A). C_T in ambient/oil-enriched treated microcosms were significantly lower compared to elevated CO_2/ oil-enriched microcosms ($p = 0.0242$) and elevated CO_2/ without oil enrichment ($p = 0.0476$) thus confirms elevated CO_2 conditions in the experiment were successfully established.

Monitored levels of pCO_2 (Figure 3.8) were much lower compared to calculated levels of pCO_2 (Appendix A), thus error in calculation of carbonate chemistry parameters such as total alkalinity (A_T), bicarbonate ions, carbonate ions, and calcite saturation were identified (Appendix A). The source of error in carbonate chemistry calculation was due

to calibration error of specialised probe used to determine pH_T in addition to recording of seawater salinity in microcosms in the beginning of the experiment and not from each sample taken at the end of experiment.

4.3.4. Hydrocarbon profile

FT-ICR-MS allows for a molecular snapshot of compositional changes between samples with resolution and identification of thousands of unique elemental composition (Smith, D. *et al.,* 2018). Complex petroleum mixtures demands high mass-resolving power, mass accuracy, and dynamic range over a wide mass to charge ratio (m/z) as a result of high heteroatom content and aromaticity (Smith, D. *et al.,* 2018). Molecular assignment is carried out when spectrum of intensity against m/z ratio is plotted. Compounds were grouped into classes according to heteroatom content (N, O, S, V, Ni) on each m/z peak. The C number for heteroatom O and heteroatom N were plotted against number of rings plus double bonds to carbon (isoabundance-contoured DBE) to produce a spectrum of molecular species of crude oil composition shown in Appendix B (N_n), Appendix C (C_cH_h), and Appendix D (O_o) that were normalised to the most abundant species of the respective heteroatom class for each mass spectrum. Double-bond equivalents (DBE) were used to identify compositional of a molecule that can be calculated due to reduction of double bond by two (Vetter, 1994; Lemkau *et al.,* 2014). Therefore, changes in any of the heteroatom classes such as the decrease in relative abundance of compounds with lower heteroatoms (hydrocarbons and N_1) is expected over time due to degradation and weathering of crude oil. With isoabundance plots, the wider analytical window allows visualisation of oil degradation and formation of new compound by obvious changes in pattern of cloud of data points on the plots.

The most abundant heteroatom classes in crude oil; compounds with one nitrogen atom (N_1) and compounds with one O heteroatom (O_1) were analysed between samples with crude oil exposed to living bacterial community and acid-killed bacterial community (killed control) to identify crude oil compounds that were biodegraded after 16 days of initial exposure to crude oil. N_1O_1 and O_2 classes were low in abundance thus not included in analysis. The spread of carbon numbers in a given DBE represent changes in alkylation. The isoabundance-contoured plots of class N_1 shows similar patterns between all four treatments (Appendix B) due to no observed change in dealkylation trend or increase in oxygen content consistent with biodegradation (Lemkau *et al.*, 2014), thus no biodegradation occurred between biological and killed control samples in both ambient and elevated CO_2 treatments. Hydrocarbon classes in samples exposed to living/acid-

killed bacteria treated with ambient/elevated CO_2 conditions were similar, suggesting hydrocarbon degradation was not observed after 16 days of crude oil exposure (Appendix C). Compounds with one oxygen atom (O_1) in crude oil exposed to living bacterial community associated to microalga showed no overall shift compared to samples exposed to acid-killed microorganisms (Appendix D).

4.4. Discussion

4.4.1. Impacts of ocean acidification and oil enrichment on microalgal community

Surface seawater collected from the FSC during the late May (spring) of 2017 were highly abundant with microflagellates (62% ± 2), diatoms (20% ± 3), coccolithophores (10% ± 2) and dinoflagellates (8% ± 1; Figure 4.2). Though extensive, the chl *a* concentration of 0.3 mg m^{-3} were relatively low compared to peak chl *a* concentration (5 mg m^{-3}) recorded in early May of the Northeast Atlantic Ocean have been reported to last up to 8 days (Debes, 2000; Friedland *et al.*, 2016). Heterotrophic microflagellates are known to be a predator of bacteria and microalgae (Suttle *et al.*, 1986). Debes, (2000) reported chl *a* concentration was normally low throughout the rest of the year. Low concentration of nutrient from post-bloom earlier in May i.e bottom up control (Debes, 2000) or grazing pressure i.e. top- down control of microalgal population were likely the reason for low chl *a* concentration when samples were collected (Behrenfeld, 2010; Friedland *et al.*, 2016). Although filtration of larger predators, such as zooplankton, was done prior to using the seawater as inoculum for the various microcosm treatments, microflagellates were not discriminated by the pore size of the filtration mesh used (120 μm). Therefore, abundance of microflagellates and ciliates in filtered seawater that were counted at the beginning of experiment suggests grazing pressure on microalgal community in addition to potential limitation in nutrients for microalgae as reduced chl *a* concentration was seen on the first two days of incubation during treatment acclimation phase of the experiment.

Impact of grazing was not assessed since growth of microflagellate population or measurement of particulate inorganic carbon and nitrogen was not carried out over time. Heterotrophic microplankton community in samples were not accounted for since only chl *a* concentration was measured as proxy to microalgal cell density and their response to the impact of oil enrichment and OA conditions. Furthermore, taxonomic identification and cell count of microplankton community was not carried out after elevated CO_2 acclimation and oil exposure to identify microplankton community shift and potential

biogeochemical consequences of increase in net community carbon consumption and net photosynthesis (Riebesell, 2004; Mackey *et al.*, 2015).

Higher CO_2 treatments and the consequential shift in carbonate chemistry positively affected the microalgal communities compared to ambient CO_2 supports reports from previous studies (Feng *et al.*, 2009; Tilstone *et al.*, 2016; Liu *et al.*, 2017). Crude oil enrichment negatively impacted chl *a* concentration of microalgae in both ambient and elevated CO_2 conditions as expected (Figure 4.3; Ozhan, Parsons and Bargu, 2014). Microalgal population recovered from oil enrichment and chl *a* concentration in microcosms treated with ambient CO_2 were in exponential phase 16 days after oil enrichment, whereas chl *a* concentration in elevated CO_2 treated microcosms were exponentially increased 3 days prior. Despite reports of low tolerance to crude oil by oceanic microalgae compared to coastal microalgal community (González *et al.*, 2009), some diatom species e.g. *Skeletonema costatum* and smaller diatoms (< 20 μm) have been shown to have high tolerance to oil pollution under low temperature (Kusk, 1978; Huang *et al.*, 2011).

4.4.2. Impacts of ocean acidification on bacterial community

Community-level analysis shows species diversity of baseline community was not affected by OA and crude oil enrichment (Figure 4.4). NMDS ordination indicated low similarity of species composition with most samples being 40% similar between treatments at all timepoints (Figure 4.5) and pairwise comparison showed species composition in all treatments showed no significant separation from control; ambient CO_2 without oil-enrichment. Taxon-level analysis showed relative abundance of *Colwellia*, *Polaribacter,* unclassified Flavobacteriaceae, unclassified *Candidatus* Pelagibacter and *Polaribacter* decreased under ocean acidification conditions (Figure 4.6). *Colwellia* was the dominating taxon in baseline community; direct sample from FSC at t0 prior to CO2 acclimation in microcosms and remained the dominating taxon under ambient/without oil treatment at t2 and t3 (Table 4.2). The genus *Colwellia* is a psychrophilic microorganism with a suite of nutrient acquisition genes that includes gaseous and aromatic hydrocarbon degradation (Gutierrez *et al.* 2013; Mason *et al.*, 2014). Despite negative impacts of elevated CO_2, *Colwellia* was tolerant to CO_2 stress and consisted of a relatively larger mean percentage of total bacterial community and under was not negatively affected by elevated CO_2/with oil enrichment. Tolerance to high CO_2 in addition to opportunistic behaviour of *Colwellia* was observed in mesocosm experiments using microbial communities from Arctic fjords (Roy *et al.*, 2013).

Significant enrichment of taxa shown in heat tree plots (Figure 4.6; Figure 4.7) by colouring on phylogenetic tree that indicates significant difference ($p < 0.05$) between median proportions of taxa for samples from different treatment determined using Wilcox rank-sum test and Benjamini-Hochberg (FDR) correction for multiple testing, while intensity of colour was relative to log2 ratio of difference in proportions (Foster, Sharpton and Grünwald, 2017) Enrichment of *Polaribacter* at t1 in ambient microcosms (Figure 4.6) corroborates to previous studies of bacterioplankton community associated with diatom dominated phytoplankton blooms in the North Sea (Gómez-Pereira *et al.*, 2012; Teeling *et al.*, 2012). *Polaribacter* was described as capable of utilising algal polysaccharides (Xing *et al.*, 2015). Therefore, it is highly likely that *Polaribacter* was directly affected by CO_2 enrichment as active growth of microalgal community were seen at t1. On the contrary, decrease in relative abundance of unclassified *Candidatus* Pelagibacter at t2 was likely due to competition with microalgae for resources during elevated CO_2 conditions as previous studies show *Candidatus* Pelagibacter were reported to decrease in abundance during diatom blooms (Teeling *et al.*, 2012).

At t2, higher diversity of taxa was enriched under elevated CO_2 compared to ambient CO_2 conditions that coincided with higher chl *a* concentration in elevated CO_2 treatments (Figure 4.6). Taxa that were enriched in elevated CO_2/without oil treated microcosms includes *Aliivibrio, Pseudomonas, Sulfitobacter,* unclassified *Vibrionaceae*, unclassified *Halomonadacea*, and unclassified *Colwelliaceae* (Figure 4.6). The enrichment of *Sulfitobacter, Acinetobacter*, and *Pseudomonas* under elevated CO_2 conditions may likely be due to symbiotic relationship with microalgal host by exchange of materials and resources, in addition to quorum sensing or signal communication (Dao *et al.*, 2018). Quorum sensing releases chemical signal molecules called autoinducer that may increase/decrease regulation of of a function based on cell density (Miller and Bassler, 2001).

Sulfitobacter, Acinetobacter and *Pseudomonas* were previously identified capable of secreting the microalgal growth hormone, indole acetic acid (Amin *et al.*, 2015; Dao *et al.*, 2018). *Sulfitobacter* is responsible for organic sulfur cycling (Ivanova *et al.*, 2004), biodegradation of algal-derived hydrocarbon (Brakstad and Lødeng, 2005), and is capable of inhibiting other taxa using sodium-channel blocking toxins (Vásquez *et al.*, 2002). *Acinetobacter* and *Pseudomonas* were characterised as nutritionally versatile and resistant to many antimicrobial compounds (Marinho *et al.*, 2009; Sharma and Thakur, 2009; Loeschcke and Thies, 2015; Shete *et al.*, 2015). *Pseudomonas* was previously described as capable of degrading microalgal cell wall components,

microalgal produced hydrocarbon, fatty acids, and triacylglycerols (Afi *et al.,* 1996) that may be in abundance during the second exponential growth phase of microalgal community at t2, and during exponential phase at t3.

4.4.3. Impacts of ocean acidification and oil enrichment on bacterial community

Bacterial diversity (H') was significantly lower when crude oil enrichment on elevated treated microcosm was compared to elevated treated microcosms without oil-enrichment for each timepoint except t3 (Figure 4.4). ANOSIM pairwise comparison of community composition showed only samples treated with OA conditions and crude oil showed low levels of separation with samples treated with OA conditions without oil enrichment, and samples treated with crude oil in ambient CO_2 conditions suggesting combined stressor has more of an effect on individual stressor. *Colwellia* was the dominating bacterial taxa in ambient conditions even with oil-enrichment, but over progression of time, it decreases in relative abundance when treated with just elevated CO_2. Surprisingly, *Colwellia* was prevalent under oil-enriched/elevated CO_2 condition. The high relative abundance of *Colwellia* in all treatments, especially in oil-enriched microcosms can be attributed to *Colwellia* potentially being hydrocarbon degraders that thrive in oil polluted environments as previously reported (Kostka *et al.,* 2011; Gutierrez *et al.,* 2013; Mason *et al.,* 2014; Garneau *et al.,* 2016). In the absence of crude oil, hydrocarbon may be available in the environment from microalgal produced hydrocarbon.

Oleispira, a hydrocarbonoclastic genera was enriched by 1% in elevated treated microcosms at 9 days after oil enrichment at t2 ($p< 0.05$) but not in oil-enriched/ambient conditions (Figure 4.7). Members of *Oleispira spp*. are obligate hydrocarbonoclastic bacteria capable of utilising linear and branched aliphatic compounds, saturated and non-saturated hydrocarbons, fatty acids, and alcohols (Yakimov, Timmis and Golyshin, 2007). The metabolic potential of *Oleispira* complements gaseous and aromatic hydrocarbon utilizing members of the hydrocarbon degrading species from the genus *Colwellia* (Mason *et al.,* 2014). This allows the specific coupling of hydrocarbonoclastic genera to successfully occupy different niches in degrading the complex mixture of hydrocarbon that makes up crude oil.

Furthermore, *Psychrobacter, Sphingopyxis,* unclassified *Sphingomonadaceae* and unclassified *Rhodobacteraceae* were found to higher in relative abundance in response to oil enrichment under elevated CO_2 conditions. *Psychrobacter,* capable of utilising *n*-alkanes (C_{13}-C_{17}) of diesel fuel (Ciric, Philp and Whiteley, 2010) was only

enriched after 17 days of oil enrichment. *Sphingopyxis spp*. have been reported capable of degrading mono- and polycyclic aromatic hydrocarbons under aerobic conditions (Godoy *et al.*, 2003; Kertesz and Kawasaki, 2010; Kim *et al.*, 2014). Unclassified *Rhodobacteraceae* was also found resilient and in high abundance to elevated CO_2 conditions as well as crude oil enrichment suggesting potential hydrocarbon degrading ability in addition to tolerance from crude oil toxicity and high CO_2 conditions. *Rhodobacteraceae* was previously reported as a key player in hydrocarbon degradation in oil contaminated beach sands of Gulf of Mexico (Kostka *et al.*, 2011).

Increase in relative abundance of *Aliivibrio* and *Marinomonas* in oil-enriched/ambient CO_2 but not in oil-enriched/elevated CO_2 ($p < 0.05$) shows tolerance to toxicity of crude oil but susceptibility to elevated CO_2 conditions (Figure 4.8). *Aliivibrio* was expected to respond negatively to oil enrichment in both CO_2 conditions as a study reported inhibition of *Aliivibrio fischeri* growth from alkylation of PAH (Kang, Lee and Kwon, 2016). However, tolerance to crude oil in this species belonging to the same genera increased relative abundance in ambient conditions but not in elevated CO_2. *Marinomonas* were found to be capable of mineralizing PAH in deep-sea sediments (Dong *et al.*, 2015). Similarly, *Polaribacter* was negatively influenced by the combined stressor of oil enrichment and high concentrations of CO_2. *Polaribacter* is a tolerant and opportunistic bacteria that was found enriched in a microcosm experiment studying hydrocarbon biodegradation of Arctic sea-ice and sub-ice microbial community (Garneau *et al.*, 2016). Elevated CO_2 conditions negatively impacted these taxa in the presence of crude oil (Figure 4.8).

Interestingly, the unclassified *GpIIa* clade belonging to the class cyanobacteria was enriched 10 days after oil-enrichment (t2) under elevated CO_2 conditions (Figure 4.7). Although some cyanobacteria have been described capable of degrading crude oil (Raghukumar *et al.*, 2001), members of *GpIIa* have not been identified as oil degraders, therefore it is likely an opportunistic bacteria that was enriched under highly stressful conditions. It is also likely that relatively higher chl *a* seen in oil-enriched elevated CO_2 treated microcosm 10 days after oil enrichment was from the increased abundance of cyanobacteria *GpIIa*. No further enrichment of cyanobacteria *GpIIa* was seen at t3 (i.e. 17 days after oil enrichment), despite an increase in chl *a* concentration. Therefore, chl *a* concentration and primary production 17 days after crude oil enrichment were dominated by microalgae population as tolerance to crude oil was higher in the eukaryotic organisms present in this study compared to the prokaryotic cyanobacteria.

4.4.4. Carbonate chemistry of subsurface seawater from FSC in microcosms

Gas mixing for the enrichment of CO_2 with ambient air that was used for elevated CO_2 treatment (730 ± 13 ppm; Figure 4.8) was slightly lower than targeted pCO_2 (750 ppm). After 2 days of CO_2 exposure, a difference of 0.2 pH units (Figure 4.9) and significant differences with C_T of control indicates exposure to elevated CO_2 affected carbonate chemistry of water similar to changes described in previous studies. Biological activity may also contribute to increasing bicarbonate concentration by secretion of organic bases for intracellular homeostasis of cells as well as formation and remineralisation of organic matter (Wolf-Gladrow, D. *et al.*, 1999; Spilimbergo *et al.*, 2005). Error in measurement of salinity prevents calculation of other seawater carbonate chemistry like bicarbonate (HCO^-_3) and carbonate ion concentrations, and calcite saturation that could further illustrate the biogeochemical consequences of OA and crude oil enrichment on microbial community found in seawater surface.

4.4.5. Biodegradation of crude oil in the oil-amended microcosms

Unlike gas chromatography analysis, conventional biomarkers nC_{17}/pristine and nC_{18}/phytane were not detectable using FT-ICR-MS due to ionization of hydrocarbon compounds for the analysis. Instead, a relatively wider analytical window of detectable compounds, compared to the conventional gas chromatography analysis such as GC-FID or GC-MS, were attained by including more volatile polar components (McKenna *et al.*, 2013; Lemkau *et al.*, 2014). Double bond equivalents (DBE) are the number of rings plus double bonds to carbon that can be used to identify compositional differences across samples (Lemkau *et al.*, 2014). Compositional images of aromaticity (DBE) vs carbon number of the three most abundant classes – i.e. N_1 (Appendix B), hydrocarbons (Appendix C), and O_1 (Appendix D), highlights compositional differences between the oil-amended treatments. Dealkylation and increasing oxygen content is expected with biodegradation of crude oil samples (Lemkau *et al.*, 2014), however the killed control samples extracted at the same time of incubation with live samples treated with different CO_2 concentrations showed no differences in these characteristics, as shown in the isoabundance-contoured plots (Appendix B, C, and D). This method is effective in analysing crude oil compounds that undergo weathering, photodegradation and biodegradation of crude oil in relatively longer timescale e.g. between 55 days to 48 months after an oil spill (Chen *et al.*, 2016; Lemkau *et al.*, 2014). This suggests that despite enrichment of bacterial taxa responsible for hydrocarbon degradation, which is

indicative of hydrocarbon biodegradation having occurred in the oil-amended microcosms, this analysis was limited by a relatively shorter incubation time.

Relative abundance of hydrocarbonoclastic bacteria such as *Oleispira* and *Marinobacter* went from undetected to a small percentage (~1%) of total bacterial community when exposed to crude oil. *Colwellia*, a potential hydrocarbon degrader, remains the dominant taxa up to 17 days after oil exposure. Since no biodegradation of hydrocarbon was detected, inhibition of hydrocarbonoclastic bacteria by persistent bacterial taxa resilient to oil enrichment is plausible. Competition for other micronutrients such as nitrogen and phosphorus were identified as a limiting factor for crude oil biodegradation as studies with additional nutrients shows improved hydrocarbon degradation by bacterial communities in soil and open ocean (Coulon *et al.*, 2007; Suja, Summers and Gutierrez, 2017). Furthermore, the cultures were maintained at low temperature (10 ^{0}C) to mimic spring temperature of FSC during sampling. Low temperature affects solubility of hydrocarbons in crude oil, thus reducing bioavailability of crude oil to hydrocarbon degraders (Hemalatha and Veeramanikandan, 2011). Sub-Antarctic mesocosm studies have shown introduction of oleophilic fertiliser and increase in temperature to 20 ^{0}C from 4 ^{0}C increased hydrocarbon-degrading microbial abundance and total petroleum hydrocarbons degradation (Coulon *et al.*, 2007).

4.5. Conclusion

Microalgal community responds positively to elevated CO_2 conditions, and recovery from crude oil pollution were shorter in time under high CO_2 treatments. Known hydrocarbon degrading bacteria were identified in FSC subsurface seawater associated to spring bacterioplankton community, however biodegradation of crude oil was not observed for up to 16 days since oil exposure. Small increase in relative abundance of hydrocarbon degrading bacteria such as *Oleispira, Marinobacter, and Halomonas* instead of blooms as of hydrocarbonoclastic bacteria after 8 to 15 days of oil exposure could be a result of competition for colimiting resources with the resilient and persistent taxa. *Colwellia*, unclassified *Gammaproteobacteria*, unclassified *Rhodobacteria*, and unclassified *Halomonadaceae* were highly abundant in simulated ocean acidification conditions and resilient to crude oil toxicity. It is hypothesisesd that these dominating and persistent taxa could inhibit hydrocarbonoclastic bacteria by competing for the same resources, or by secreting antimicrobials that inhibit growth of the hydrocarbonoclatic population. Therefore, microbial community from the subsurface of FSC during spring has the potential to recover from an oil spill under future ocean conditions (750 ppm),

however the dynamics of the microbial community dictates the recovery of the ecosystem from crude oil pollution. A major factor that could be linked to inhibition of biodegradation of crude oil after 17 days is competition for resources with persistent and dominating heterotrophic taxa that is closely associated with the success of the microalgal community.

Chapter 5: Impacts of ocean acidification and crude oil pollution on fall bacterioplankton community (Faroe-Shetland Channel)

5.1. Introduction

In Chapter 4, microalgal-bacterial consortia sampled from the Faroe-Shetland Channel (FSC) during spring were exposed to elevated CO_2 and crude oil pollution. Microbial population dynamics can vary seasonally due to stratification or deepening of layers, light-dark regiment temperature, and weather patterns (Longhurst, 1995; Martinez *et al.*, 2011). Northeast Atlantic spring and fall blooms have been recorded and described since 1980s (Colebrook, 1982; Longhurst, 1995; Dandonneau *et al.*, 2004; Lévy *et al.*, 2005; Martinez *et al.*, 2011). The diatom dominated spring microalgal bloom in the North Atlantic Ocean is the most productive period to occur annually within the region (Ward and Van Oostende, 2016). Unlike spring blooms that are initiated due to stratification of upper layer of the seawater mass at the end of winter, fall blooms occur due to nutrient entrainment and deepening of the mixed layer in the end of summer in addition to reduction in grazing pressure as mesozooplankton migrates towards deep waters (Colebrook, 1982; Martinez *et al.*, 2011).

Initial reports of spring and fall blooms were similar in amplitude before the 1980s, however in the beginning of 2000, weaker blooms were reported on the eastern Atlantic (east of 40^0 W). This was due to delayed deepening of the mixed layer at the end of summer (Martinez *et al.*, 2011). This delay is attributed to climate change, where increasing levels of global atmospheric CO_2 affects warming and wind patterns. In addition to weakening fall blooms in the north-eastern Atlantic, climate change has recently been reported to shift the phenology of Arctic region from single annual blooms to two-phase annual blooms that occur first in the spring then again in the fall. This phenomenon was described as a shift from polar mode to temperate mode for the Arctic region (Uchimiya *et al.*, 2016).

Chl *a* concentration during fall phytoplankton blooms have been shown to be tightly coupled to bacterial abundance and production despite decrease in seawater temperature (Uchimiya *et al.*, 2016). Therefore, the aim of this chapter was to investigate the potential response of microbial community sampled during fall to ocean acidification and crude oil pollution. Fall microbial community from the same site (Faroe-Shetland Channel) that was sampled in Chapter 4 provides an opportunity for a seasonal comparison (between fall and spring) of the microbial community response to crude oil pollution under ocean acidification conditions.

5.2. Methods

5.2.1. Field sampling

Surface seawater (5m) from the Faroe Shetland Channel (FSC) were collected on October 8, 2017 using an array of Niskin bottles attached to a rosette sampler coupled with CTD as described in Chapter 2. Satellite observations showed chl *a* distribution was scattered in the FSC and chl *a* concentration were low to zero in the site of interest (Figure 5.1). During sampling, the temperature of subsurface seawater at 5 m depth was recorded at 11.33 ^{0}C and salinity of 34.628. Filtration of 20 L of subsurface seawater with a nylon mesh of 120 µm pore size was performed to remove zooplankton and stored in autoclaved polypropylene carboy. Triplicates of 50 ml of the homogenised phytoplankton community were preserved in Lugol's iodine and sent to Marine Scotland for taxonomic identification and enumeration (Dr. Pablo Diaz, Marine Scotland).

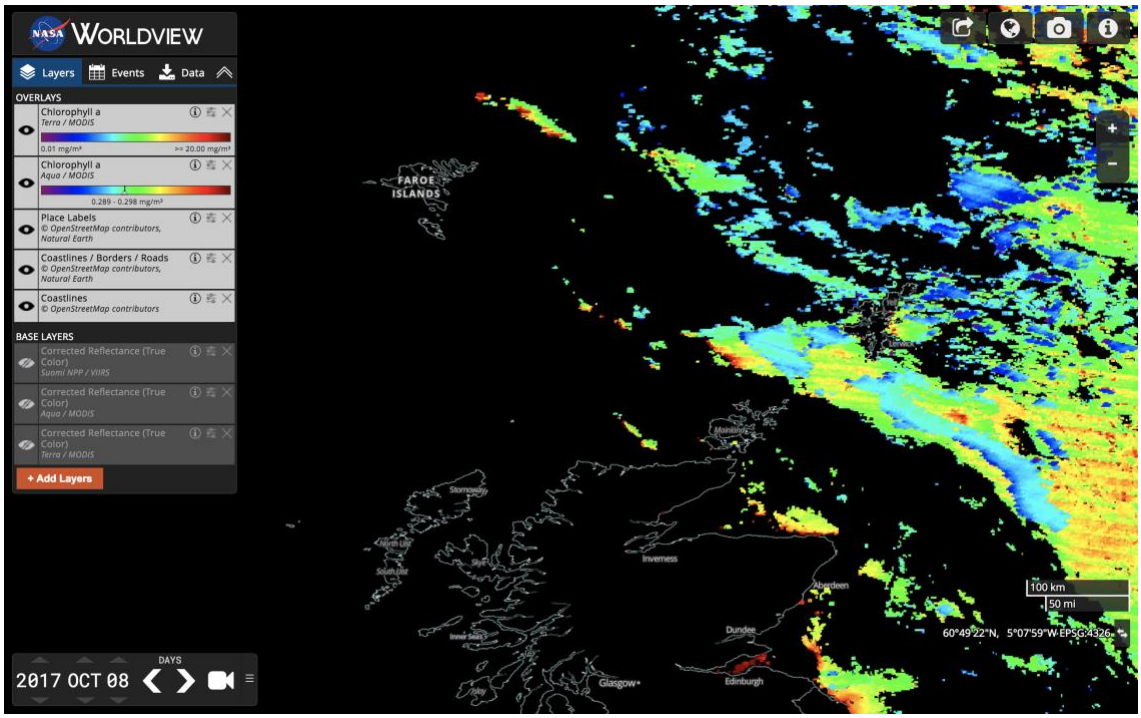


Figure 5.1. Satellite observation of chlorophyll *a* abundance in the FSC region during collection of seawater samples fall of 2017 (NASA Worldview, 2018). Chl *a* concentration of 0.289 – 0.298 mg/m3 observed by Aqua/MODIS closest to sampling site (60^{0}49'22"N, 5^{0}07'59"W) on the day of sampling.

5.2.2. Microcosm setup

Subsurface seawater that were filtered with 120 µm sieve were homogenised by mixing in a 10 L pre-sterilised polypropylene carboy before aliquoting 400 ml into microcosms. Microcosms were treated with 1) FSC subsurface seawater; biological

control, 2) acid-killed/oil-enriched FSC subsurface seawater; hydrocarbon analysis control, 3) oil-enriched FSC subsurface seawater; biological analysis, and 4) oil-enriched FSC subsurface seawater; hydrocarbon analysis. Each treatment was carried out in triplicates. Microcosms were maintained at 11 ^{0}C using a water bath connected to a thermostat-enabled cooler to simulate temperature of sampling location and acclimated to ambient CO_2 (400 ppm) or elevated CO_2 (750 ppm). After 5 days of CO_2 acclimation, 1 % (v/v) Schiehallion crude oil were added to the microcosms as detailed in section 2.2.3 of Chapter 2.

5.2.3. Chl a abundance and pH readings

Samples were taken on day 2, 4, 7, 11, 14, 18, and 22 for measurement of pH and for chl *a* extraction. The pH readings were taken immediately after each sampling, while 2 ml of samples were centrifuged to pellet cells and stored in 90% acetone at -20 ^{0}C prior to chl *a* fluorometric analysis as described in Chapter 2 (section 2.3.1). Average readings were analysed using two-way ANOVA and Tukey's multiple comparison test to determine significance of treatments on chl *a* and pH.

5.2.4. Bacterial community analysis

Samples were taken for DNA extraction at the following 4 timepoints: 1) Prior to acclimation in microcosms in order to obtain a baseline reference for the bacterial community of the FSC in the fall of 2017 (t0); 2) 5 days after CO_2 acclimation (t1); 3) day 15 (t2), or 10 days of oil enrichment; 4) day 22 (t3) or 17 days of oil enrichment. At each sampling point, 5 ml of homogenised sample was taken from each microcosm, filtered on 0.22 μm polycarbonate membrane filters and then stored at -20 ^{0}C prior to DNA extraction and subsequent Illumina MiSeq sequencing analysis, as described in section 2.3.2 (Chapter 2). These samples were pooled together with those from Chapter 4 for the Illumina MiSeq sequencing at Edinburgh Genomics, thus allowing for unbiased comparison between the spring and fall bacterial communities in these experiments performed across these two seasons in 2017. Sequencing data were analysed using mothur according to the pipeline detailed in section 2.3.2 (Chapter 2). Alpha diversity was analysed using Shannon H' indices and NMDS plots of log transformed Bray Curtis similarity index. Heat tree diagrams were plotted using metacodeR to compare significant differences in relative abundance of taxons from two treatments described in section 2.3.2 (Chapter 2).

5.2.5. Carbonate chemistry analysis

Subsurface seawater from FSC during fall were transferred immediately from Niskin bottles and samples from microcosms at the end of experiment were preserved in 100 ml narrow necked glass bottles with minimal headspace and sealed with silicon free grease (Apiezon) after preserving with a final concentration of 0.02% mercuric chloride according to SOP 1 of Guide to Best Practices for Ocean CO_2 Measurements (Dickson, Sabine and Christian, 2007). Bottles containing samples were stored at room temperature and in the dark. Prior to analysis, sealed bottles were placed in a 25 ^{0}C water bath. Total dissolved inorganic carbon (DIC) was determined using acidification, gas stripping and infrared detection followed by total hydrogen ion analysis described in section 2.3.4 (Chapter 2).

5.2.6. Hydrocarbon extraction and analysis

Total hydrocarbon extraction of microcosms that were untouched throughout the experiment was carried out using HPLC-grade dichloromethane as described in section 2.3.3 (Chapter 2). Samples were then stored at -20 ^{0}C in amber glass vials with Teflon caps prior to analysis. Atmospheric pressure photoionisation (APPI) coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) was carried out by Dr. Faye Cruikshank from the School of Chemistry, University of Edinburgh (Lemkau *et al.*, 2014; McKenna *et al.*, 2013).

5.3. Results

5.3.1. Characterisation of microalgal community

Triplicates of 50 ml of seawater samples that were filtered and fixed in Lugol's iodinde were enumerated and taxonomically identified. The total cell density of the phytoplankton community was found to be 4607 ± 1023 cells/L. The community was dominated by dinoflagellates (84%) followed by ciliates (11%), diatoms (4?%), and others (<1%) (Figure 5.2). No coccolithophores or microflagellates were identified in this fall phytoplankton community. Compared to the spring community, phytoplankton cell concentrations were significantly lower ($p = 0.0035$) but a difference of 0.37 ± 0.21 of chl *a* concentration not significantly different ($p = 0.09$). The difference in cell concentration is greater (239,194 ± 14,441 cells/L) due to presence of microflagellates in spring community. Dinoflagellates from surface water of FSC sampled during fall were found to be less abundant than diatom dominated spring bloom samples (Chapter 4).

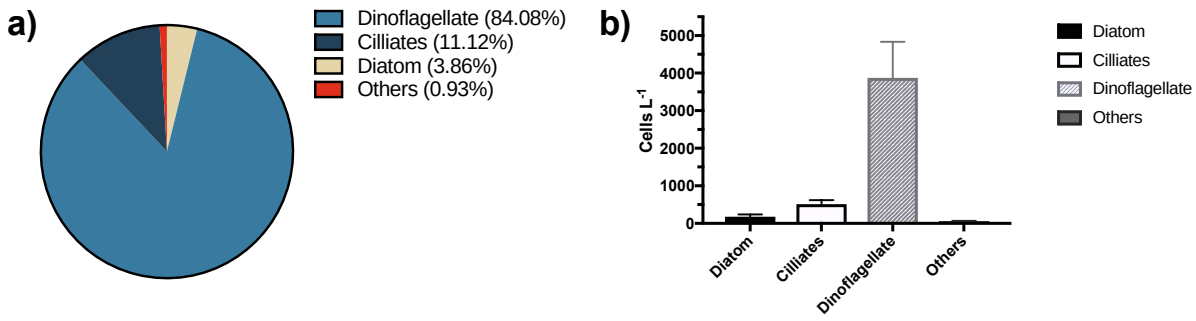


Figure 5.2. a) Composition of microalgal community shown in mean percentage of total microalgal community collected from the surface seawater of the FSC in the northeast Atlantic during the fall in October of 2017. b) Cell concentrations (per litre) of major microalgal groups and ciliates identified in the fall surface seawater from the FSC. Values are mean of samples (n=3) and error bars show standard error of mean.

Table 5.1 shows the taxonomic identity of various members of the phytoplankton community that could be identified, with athecate dinoflagellates dominating (up to 50%) of the total community. Up to 15 species of dinoflagellates were identified. Diatoms that comprise almost 4% of the phytoplankton community were dominated by *Pseudo-nitszchia spp*.

Table 5.1. Summary of taxonomic analysis of microplankton community of FSC subsurface seawater sampled during fall. Values are mean % (n = 3) of taxon ± standard error of mean.

Lineage	Taxa	Mean % ±SEM
Dinoflagellate	Unidentified athecate dinoflagellate (10 - 20 µm)	38.97 ± 20.31
	Unidentified athecate dinoflagellate (20 - 30 µm)	12.03 ± 5.39
	Unidentified thecate dinoflagellate (20 - 30 µm)	5.86 ± 4.95
	Ceratium lineatum	3.23 ± 0.46
	Gymnodinium spp. 20 - 30 µm	2.93 ± 1.77
	Amphidinium spp.	2.92 ± 0.28
	Torodinium robustum	2.46 ± 0.80
	Gyrodinium spp. 30 - 40 µm	2.31 ± 0.28
	Gyrodinium spp. 20 - 30 µm	2.02 ± 1.02
	UI athecate dinoflagellate 30 - 40 µm	1.82 ± 1.59
	Mesoporos spp.	1.54 ± 0.14
	Gyrodinium spp. 40 - 50 µm	1.23 ± 0.16
	Gymnodinium spp. 30 - 40 µm	0.94 ± 0.72
	Katodinium glaucum	0.77 ± 40
	Gyrodinium spp. > 50 µm	0.77 ± 0.15
	Azadinium spp.	0.76 ± 0.54
	Protoperidinium spp 10 -30 µm	0.61 ± 0.41
	Gymnodinium spp. 40 - 50 µm	0.61 ± 0.40
	Ceratium fusus	0.47 ± 0.27

Continued from Table 5.1

Lineage	Taxa	Mean % ± SEM
	UI thecate dinoflagellate 10 - 20 µm	0.46 ± 0.26
	Protoperidinium steinii	0.30 ± 0.25
	Dinophysis acuminata	0.16 ± 0.16
	Scrippsiella spp.	0.16 ± 0.16
	Unidentified athecate dinoflagellate 40 - 50 µm	0.16 ± 0.16
	Amphidinium crassum	0.15 ± 0.15
	Gymnodinium spp. 10 - 20 µm	0.15 ± 0.15
	Karenia mikimotoi	0.15 ± 0.15
	Protoperidinium claudicans	0.15 ± 0.15
Diatom	*Pseudo-nitzschia spp.*< 5 µm	1.24 ± 0.62
	Chaetoceros spp. >20 µm	0.77 ± 0.56
	Chaetoceros spp. 15 - 20 µm	0.63 ± 0.63
	Corethron hystrix	0.31 ± 0.31
	Thalassiosira spp. 15-20 µm	0.31 ± 0.31
	Pseudo-nitzschia spp. >5 µm	0.30 ± 0.30
	Centric diatom 20-30 µm	0.15 ± 0.15
	Centric diatom 40-50 µm	0.15 ± 0.15
Silicoflagellate	*Dictyocha speculum*	0.93 ± 0.47

5.3.2. Microalgal response to ocean acidification and oil pollution

The microalgal community from subsurface seawater of the FSC sampled during the fall of 2017 was acclimated to ambient (400 ppm) and elevated CO_2 (750 ppm) for 5 days. Elevated CO_2 treatments initially reduced chl *a* by day 2, but chl *a* concentration increased by day 4 (Figure 5.3). Based on a 2-way ANOVA and Tukey's multiple comparison test, no significant differences were determined for chl *a* between treatments up to day 4, despite higher levels of chl *a* under elevated CO_2 conditions. Oil enrichment on day 5 negatively affected chl *a* concentration in both ambient and elevated CO_2 treated microcosms, as seen by reduced chl *a* concentration on day 7.

Death phase of microalgae seen as decline in chl *a* concentration was seen in all treatments after day 4 (Figure 5.3). Significant differences in chl *a* concentration were

detected in the elevated CO_2 treatments by day 7 in the presence and absence of crude oil ($p = 0.0324$). Higher chl *a* concentration were observed in the elevated CO_2 treatment without oil by day 7, though followed by a rapid decline in these concentrations by day 11. Decline in microalgal concentration were slower in ambient treated microcosm without oil enrichment despite relatively lower chl *a* concentration on day 7. Average chl *a* concentration were not significantly different between all treatments on day 11 ($p < 0.05$). Samples treated with oil were still in exponential decline phase on day 14, while ambient/without oil treated microcosms were in stationary phase. Chl *a* of ambient CO_2 treated microcosms were significantly different ($p= 0.0367$) between oil-enriched and the oil untreated controls on day 14.

Surprisingly, the micro-algal community in microcosms treated with elevated CO_2 were in exponential growth phase on day 14 (Figure 5.3). On day 18, average chl *a* concentration of ambient oil-enriched microcosm was significantly lower than those in the ambient oil-untreated controls ($p =0.014$), and in the elevated CO_2 oil-untreated microcosm ($p = 0.0402$). At the point of termination of these experiments (day 22), no significant differences in chl *a* concentration were identified across all of the treatments.

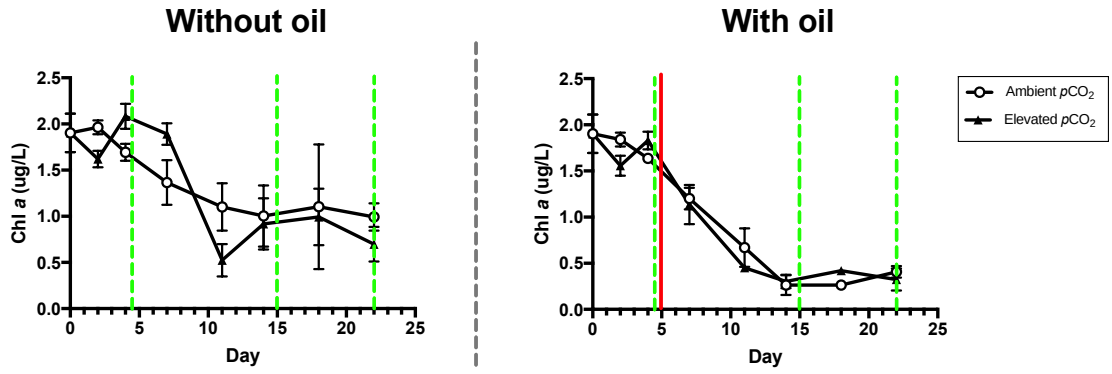


Figure 5.3. Growth response of microalgae of microcosms treated with ambient (open circles) and elevated CO_2 (closed triangle) in no-oil control (left) and with oil enrichment (right). Oil was added on day 5 (indicated by red lines), while samples for 16S rRNA sequencing were taken at three timepoints (indicated by green lines): t1 (day 4 prior to oil enrichment), t2 (day 15), and t3 (day 22).

5.3.3. Bacterial community response

Similar to previous chapters, Shannon diversity index (H') was used to assess alpha-diversity or abundance and evenness of population within the community of the baseline community (t0) treated with CO_2 and crude oil-enriched microcosms. H' of the baseline community were 2.3 ± 0.1 (Figure 5.4). Average of H' assigned to each treatment

was not significantly different when assessed with Dunnett's multiple comparison 2-way ANOVA test ($p < 0.05$).

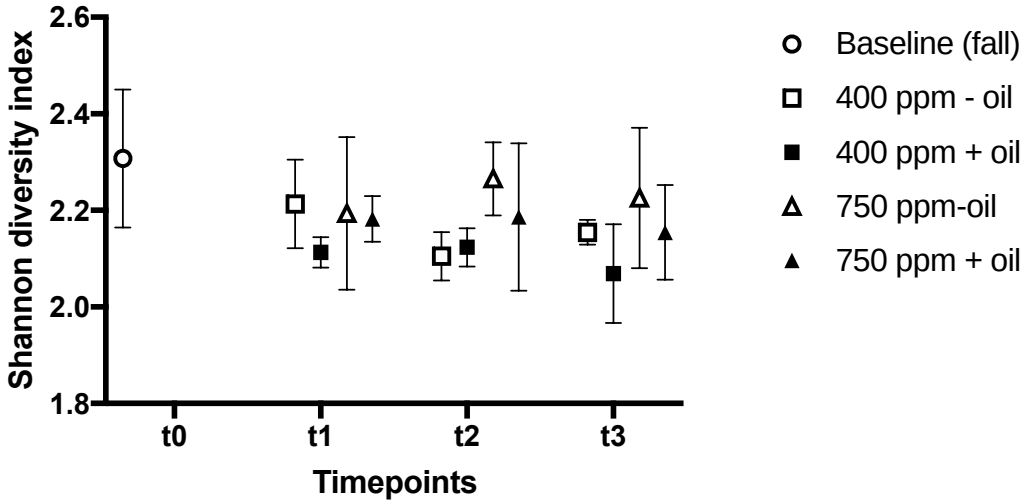


Figure 5.4. Shannon diversity index of bacterial community between samples in different treatments over time. Values are mean of triplicates (n = 3) and error bars show standard error of means (SEM) of each sample.

Beta-diversity or dissimilarity between the species composition under different treatments were assessed using Bray-Curtis similarity index normalised to log (X + 1). NMDS plot shows species composition of bacterial community between all samples treated with ambient/elevated CO_2, with/without oil enrichment, at three different timepoints were at least 35% similar with species composition of most samples being 50% similar to each other (Figure 5.5). ANOSIM pairwise test was carried out and identified separation of ambient oil enriched (400 ppm + oil) species composition with ambient CO_2 without oil enrichment (400 ppm – oil; R statistics 0.593, $p = 0.02$) and oil-enriched elevated CO_2 treatment (750ppm + oil; R statistics 0.278, $p = 0.02$). Triplicates of baseline samples were 50% similar (Figure 5.5). No obvious separation of samples under combined stressors were observed other than ambient/no-oil treated samples at t1 and t2 were mostly 60% similar while elevated CO_2/no-oil samples at t2 and t3 were 60% similar.

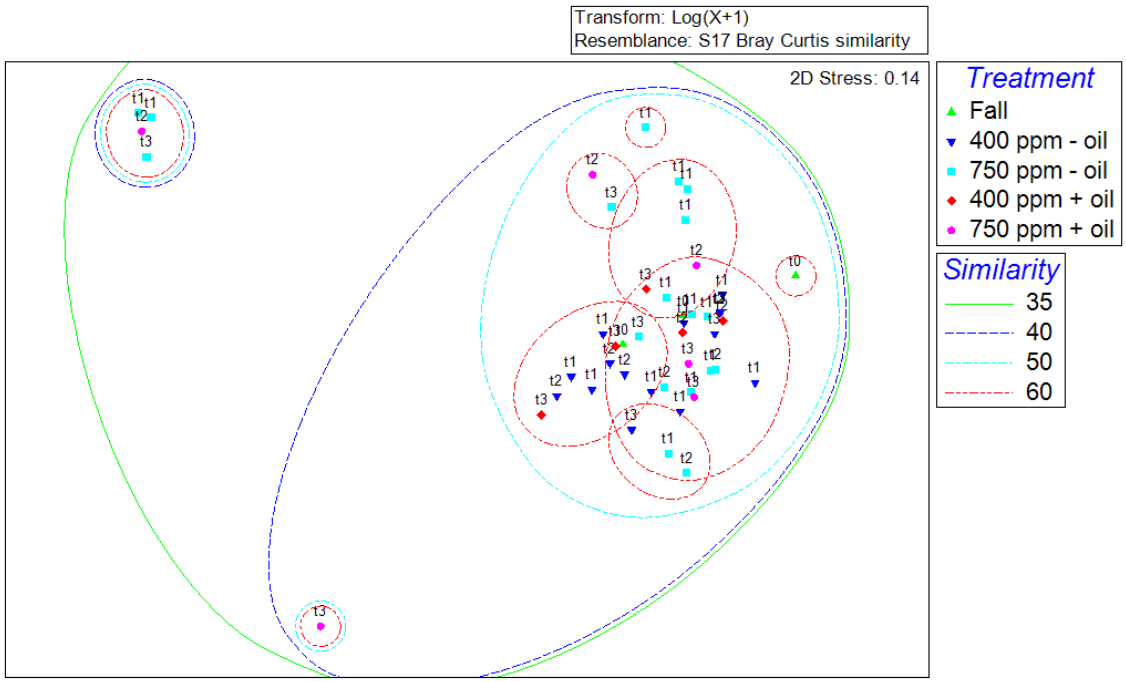


Figure 5.5. NMDS plots based on Bray Curtis similarity index of the bacterial community associated with the fall microalgal community in microcosms treated with ambient/elevated CO_2, and with/without oil enrichment at four timepoints (t0 representing the baseline prior to culturing; t1representing day 4 prior to oil enrichment; t2 representing day 15; and t3 representing day 22). Treatments are represented by coloured shapes shown in legend where 'Fall' is the baseline sample at t0; '400 ppm' or '750 ppm' is the CO_2 concentration (ambient or elevated respectively), and '+ oil' or '- oil' is the presence or absence of oil respectively. Clusters based on log (X + 1) Bray-Curtis similarity index (%) are indicated by coloured ellipses.

Species richness of 82 operational taxonomic units (OTUs) were identified in the MiSeq 16S rRNA gene libraries representing the bacterial community associated with the surface seawater from the FSC when exposed to ocean acidification conditions (Figure 5.4). Interestingly, only 3 less OTUs (79 in total) were identified in the combined stressor of ocean acidification and crude oil exposure. Removal of rare species (5 OTU's or less) was done prior to transformation of data by Wilcox rank-sum test and Benjamini Hochberg (FDR) to avoid sampling depth bias (Figure 5.6 and 5.7; Foster, Sharpton and Grünwald, 2017). Only significantly enriched taxa in respective conditions determined by Wilcox rank-sum test and Benjamini Hochberg (FDR) correction for multiple comparisons were shown in smaller heat trees (Figure 5.6 and 5.7).

Baseline microbial community collected in fall

Colwellia were the dominant group in baseline community (19% ± 5%) as well as in the bacterial community of microcosms acclimated with ambient (19% ± 3%) and elevated CO_2 (19% ± 3%) treatments at t1 (Table 5.2). The baseline community also comprised members of unclassified bacteria (9% ± 2%), unclassified *Gammaproteobacteria* (9% ± 2%), unclassified *Rhodoproteobacteraceae* (7% ± 2%), unclassified *Flavobacteriacea* (4% ± 1%), unclassified *Alteromonadaceae* (4% ± 2%), *Polaribacter* (3% ± 2%), unclassified *Candidatus* Pelagibacter (3% ± 1%), *Aliivibrio* (3% ± 2%), unclassified *Proteobacteria* (2% ± 1%), *Psychrobacter* (2% ± 1%), *Marinobacter* (2% ± 2%), unclassified *Colwelliaceae* (2% ± 0%), unclassified *Alteromonadales* (2% ± 1%), unclassified *Proteobacteria* (2% ± 1%), and unclassified *Vibrionaceae* (2% ± 1%). *Pseudomonas*, *Sulfitobacter*, *Tenacibaculum*, *Pseudoalteromonas*, unclassified *GpIIa*, unclassified *Erythrobacteraceae*, unclassified *Rhodospirillaceae*, unclassified *Betaproteobacteria*, and *Marinomonas* represented about 1% ± 0% of the total bacterial community (Table 5.2).

Bacterial community comparison after 5 days of elevated CO_2 treatment

Significant differences in median proportion of ratio of relative abundance for taxa ($p<0.05$) indicated by coloured branches on the heat tree diagram shows enrichment of *Psychrobacter*, *Erythrobacter*, unclassified *Bacillariophyta*, and unclassified *Candidatus* Pelagibacter in elevated CO_2 conditions compared to ambient conditions at t1 (Figure 5.6). *Psychrobacter* in elevated CO_2 treated conditions (5% ± 2) were much higher than in ambient treated conditions (1% ± 0; Table 5.2). The enrichment of *Erythrobacter* unclassified *Candidatus* Pelagibacter and unclassified *Bacillariophyta* were < 1% of relative abundance of total bacterial community. Bacterial community that were not as successful in elevated CO_2 conditions were determined by the enrichment of ambient treated microcosms at t2 compared to elevated CO_2 communities of the same timepoint.

Unclassified *Rhodobacteraceae*, unclassified *Alteromonadaceae*, unclassified *Gammaproteobacteria*, *Aliivibrio*, *Marinobacter*, *Marinomonas*, *Polaribacter*, unclassified *Alteromonadales*, unclassified *Oceanospirillaceae*, unclassfied *Vibrionaceae*, and unclassified *Flavobacteriaceae* were enriched in the ambient CO_2 treated microcosms at t1 compared to elevated CO_2 conditions thus their enrichment was inhibited under elevated CO_2 conditions (Figure 5.6). Relative abundance of unclassified

Rhodobacteraceae and unclassified *Alteromonadaceae* were reduced by 4% in elevated CO_2 treated microcosms (Table 5.2).

Unclassified *Gammaproteobactria* decreased by 2% while unclassified *Flavobacteriaceae,* unclassified *Alteromonadales* and *Marinomonas* decreased by 1%. Unclassified *Oceanospirillaceae, Aliivibrio,* and *Marinobacter* reduced by 1% thus making these taxa undetected under elevated CO_2 treatment (Table 4.2).

Bacterial community after 15 days of elevated CO_2 treatment

Despite previous decrease in relative abundance under elevated CO_2 treatment, *Aliivibrio* and *Marinomonas* were enriched on day 15 (t2; Figure 5.6). Previously undetected *Aliivibrio* and *Halomonas* were enriched by 1% of total bacterial community under elevated CO_2 conditions. Other taxa that were enriched when treated with elevated CO_2 includes unclassified *Halomonadaceae,* unclassified *Colwelliaceae, unclassified Proteobacteria,* unclassified *Candidatus* Pelagibacter, *Pseudomonas, Salinimonas, Sulfitobacteria,* and unclassified *Betaproteobacteria* (Figure 5.6). Unclassified *Halomonadaceae* were 4% higher in elevated CO_2 conditions at t2 than ambient CO_2 conditions. Unclassified *Colwelliaceae* were 2 % higher in elevated CO_2 (3 % ± 0) than ambient CO_2 (1% ± 1) at t2. Unclassified *Candidatus* Pelagibacter were 2% higher in elevated CO_2 conditions and unclassified *Betaproteobacteria* were 1% more enriched in elevated CO_2 conditions at t2. Decrease in relative abundance of unclassified *Alteromonadaceae* (4 - 10%), *Polaribacter* (2 - 4%), unclassified *Alteromonadales* (1 - 4%), and *Marinobacter* (1%) were seen under elevated CO_2 condition (Figure 5.7; Table 5.2).

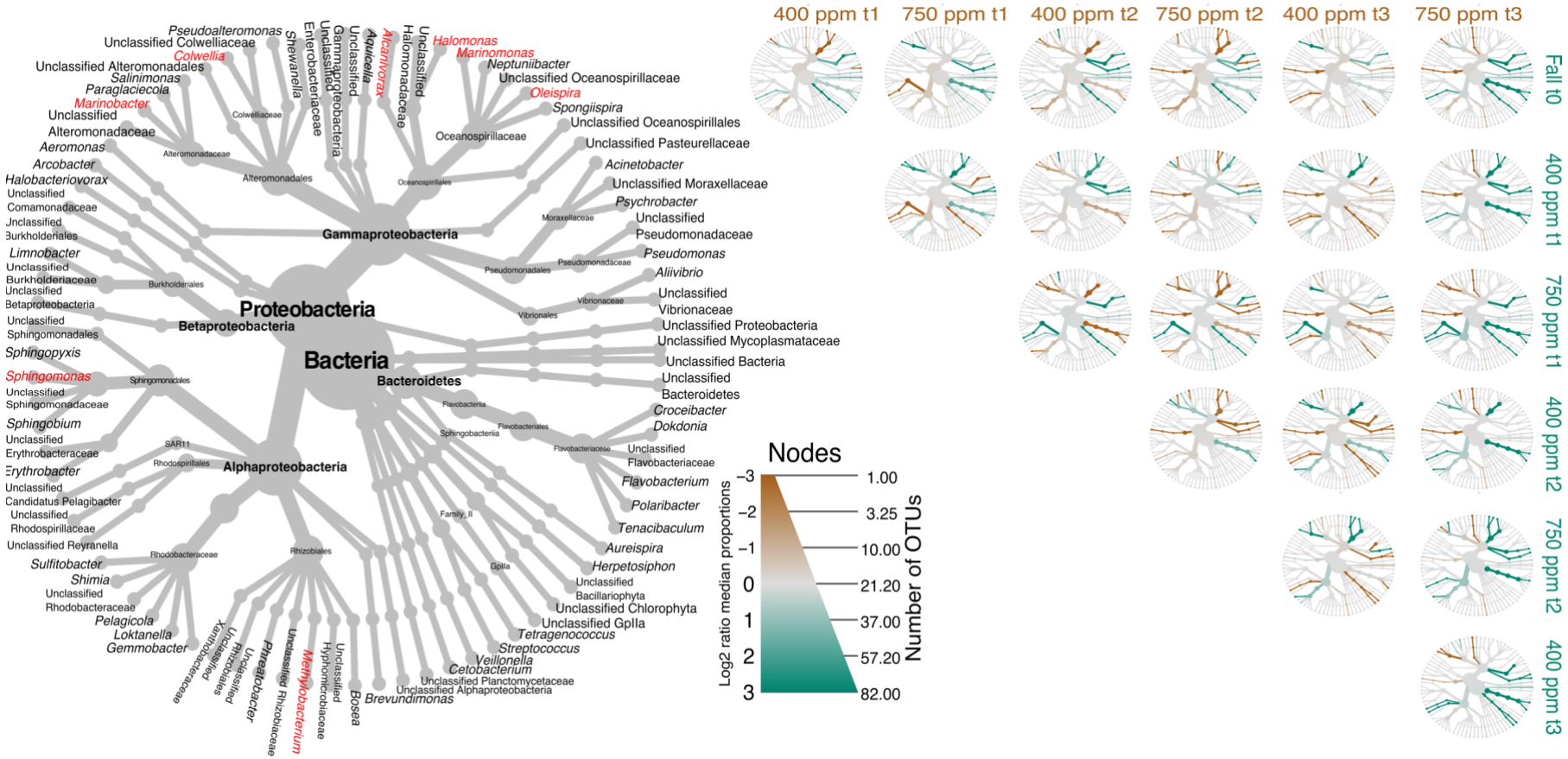


Figure 5.6. Heat tree showing pairwise comparison of the microbial community exposed to ambient and elevated CO_2 treatments at different timepoints. The larger tree on left displays all the OTUs identified, including related taxa of cultivated species with known hydrocarbonoclastic potential (shown in red). The diameter of nodes on the larger tree corresponds to qualitative number of OTU's in the respective taxa. Smaller trees represent significantly enriched taxa between paired treatments; ambient (400 ppm) or elevated CO_2 (750 ppm) at different timepoints corresponding to horizontal (brown) and vertical (green) axis. Sampling timepoints were t0 (the baseline in-situ community); t1 at day 4; t2 at day 15; t3 at day 22. Intensity of colour in smaller trees (brown or green) corresponds to difference in ratio of relative abundance of taxa in paired treatments.

Bacterial community after 22 days of elevated CO_2 treatment

At the end of experiment (day 22), bacterial community in elevated CO_2 treated microcosms showed increased relative abundance of unclassified *Alteromonadaceae, Marinobacter,* unclassified *Betaproteobacteria,* and unclassified *Erythrobacteraceae* compared to ambient CO_2 treated microcosms (Figure 5.6). Average percentage of unclassified *Alteromonadacea* were 2 - 8% higher in elevated CO_2 conditions, while *Marinobacter* was 1 – 2% higher at elevated CO_2 treated microcosms, and unclassified *Betaproteobacteria* were 1% higher at the same treatment (Table 5.2).

On the contrary, *Aliivibrio, Polaribacter,* unclassified *Alteromonadales,* unclassified bacteria, unclassified *Colwallaceae,* unclassified *Flavobacteriaceae,* unclassified *Gammaproteobacteria,* unclassified *Proteobacteria,* unclassified *Vibrionaceae,* and *Moraxellaceae* (family) were less successful in elevated CO_2 conditions as these taxa were relatively more abundant in ambient treated microcosms compared to elevated treated microcosm at the end of experiment (Figure 5.6). *Aliivibrio* made up 4% ± 2 of mean percentage of total bacterial community in ambient CO_2, while in elevated CO_2 conditions, *Aliivibrio* were <1 % of mean percentage of the total bacterial community. Unclassified *Gammaproteobacteria,* decreased by 5 – 7, unclassified bacteria decreased by 1 - 5%, unclassified *Colwelliaceae* decreased by 2 – 5%, unclassified *Flavobacteriaceae* decreased by 1 – 4%, unclassified *Vibrionaceae* decreased by 2 - 4% , and %, unclassified *Alteromonadales* decreased by 2% in elevated CO_2 treatments at t3 (Table 5.2).

Bacterial community response after 10 days of oil-enrichment in elevated CO_2 condition

Impact of oil-enrichment after 10 days under elevated CO_2 condition increased relative abundance of 4 taxa while ambient CO_2 treatment enriched 7 taxa (Figure 5.7). Relative abundance of *Alcanivorax* were 2% ± 1 in 750 ppm CO_2 but was undetected in 400 ppm CO_2 (Table 5.2). Mean percentage of *Psychrobacter* were 2 – 8% higher in oil-enriched/elevated CO_2, while unclassified *Betaproteobacteria* and unclassified *Sphingomonadaceae* were 1 – 2% higher in oil-enriched/elevated CO_2 (Table 5.2).

Furthermore, mean percentage of taxa that were negatively affected by elevated CO_2 after initial exposure to crude oil pollution at t2 were *Pseudoalteromonas*, unclassified *Gammaproteobacteria, Aliivibrio,* unclassified *Vibrionaceae,* unclassified *Flavobacteriaceae, Polaribacter, Cetobacterium*, and unclassified *Candidatus* Pelagibacter (Figure 5.7). Comparison between mean percentage of oil-enriched/elevated

CO_2 with oil-enriched/ambient CO_2 at t2 showed *Pseudoalteromonas* were less than 1% of mean percentage of bacterial community in oil-enriched/elevated CO_2 while the average percentage of total bacterial community in oil-enriched/ambient treated microcosms were higher (1% ± 0), unclassified Gammaproteobacteria in oil-enriched/elevated CO_2 (13% ± 5) were lower than oil-enriched/ambient CO_2 (16% ± 1; Table 5.2). Mean percentage of *Aliivibrio* and unclassified *Vibrionaceae* in oil-enriched/ambient treated microcosms were 3% higher, unclassified *Flavobacteriaceae* was 2 – 3%, *Polaribacter* 1% higher, *Cetobacterium* 0 – 1% more, and unclassified *Candidatus* Pelagibacter 0 – 4% higher compared to oil-enriched/elevated CO_2 (Table 5.2).

Bacterial community response after 17 days of oil-enrichment in elevated CO_2 condition

When relative abundance of bacterial community in oil-enriched/ambient CO_2 treated microcosms were compared with oil-enriched/elevated CO_2 treated microcosms at t3, unclassified *Gammaproteobacteria, Shimia,* unclassified *Colwelliaceae*, *Tenacibaculum, Sulfitobacter,* unclassified *Vibrionaceae* unclassified *Candidatus* Pelagibacter, *Pseudomonas,* and unclassified *Halomonadaceae* were prevalent under elevated CO_2 conditions while *Colwellia, Marinobacter,* unclassified *Alphaproteobacteria*, unclassified *Alteromonadaceae,* unclassified *Alteromonadales,* unclassified bacteria, and the phylum *Cyanobacteria* were negatively affected by elevated CO_2 and 17 days of oil enrichment (Figure 5.7). *Aliivibrio* was negatively impacted by elevated CO_2/oil-enriched treatment at t2 increased in compared to elevated/oil-enriched treatment at t3. (Figure 5.7).

Increase in relative abundance of unclassified *Gammaproteobacteria* by 5 – 15%, and unclassified *Halomonadaceae* by 1 – 13% were relatively higher compared to other taxa (Table 5.2). *Shimia* and unclassified *Colwelliaceae* increased by 1 – 2%, while *Aliivibrio, Tenacibaculum, Sulfitobacter,* and unclassified *Vibrionaceae* increased by 1% in oil-enriched/elevated CO_2 treated microcosms compared to oil-enriched/ambient CO_2 microcosms (Table 5.2). A large decrease in relative abundance of *Colwellia* (10 – 32%) in elevated CO_2 treatments was observed. Relative abundance of unclassified bacteria by 1 – 3% were identified under higher CO_2 treatments while *Marinobacter,* unclassified *Alphaproteobacteria* and unclassified *Alteromonadales* decreased by 1% was observed under elevated CO_2 treatment (Table 5.2).

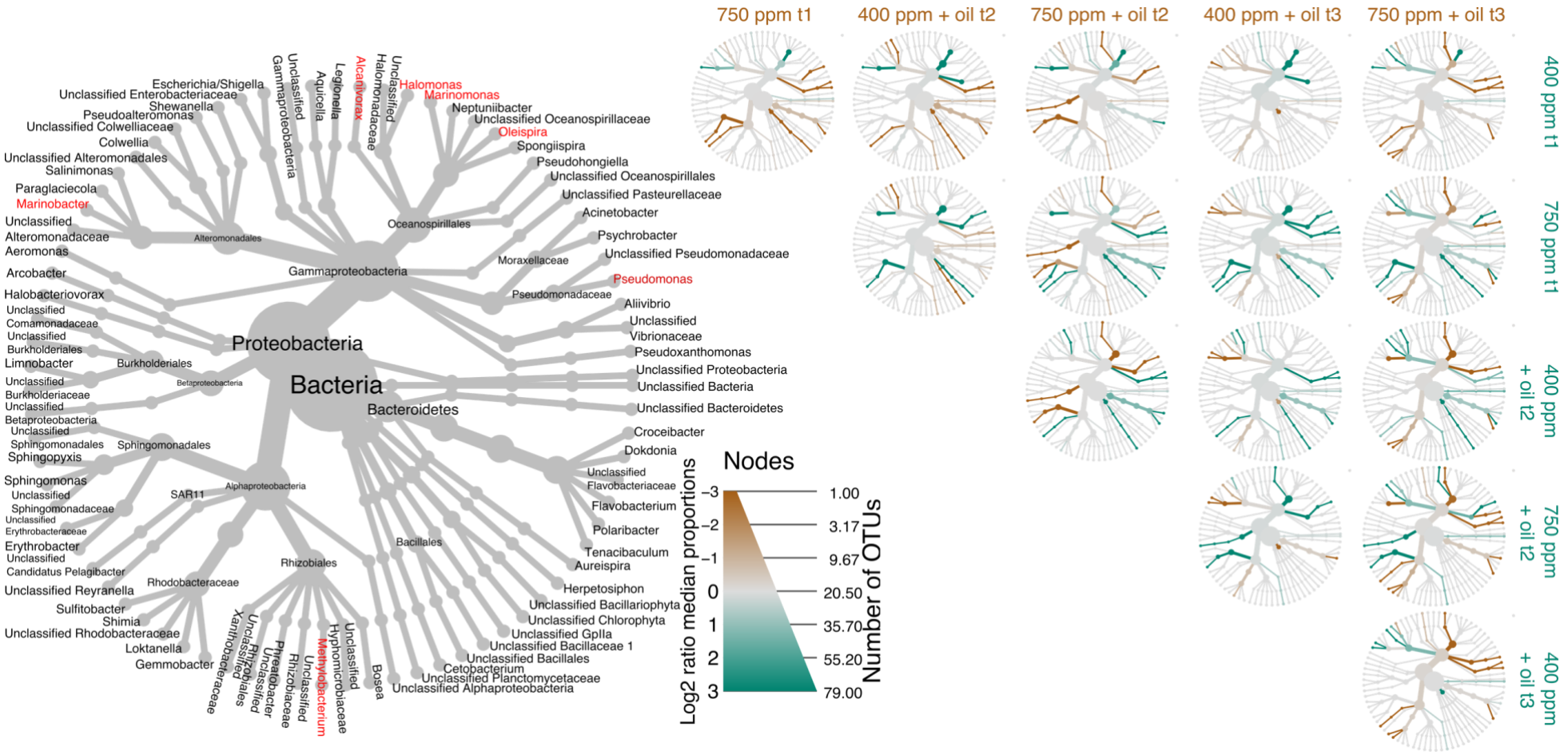


Figure 5.7. Heat tree showing pairwise comparison of the microbial community exposed to crude oil enrichment in ambient (400 ppm) or elevated CO_2 (750 ppm) treatments at different timepoints. The larger tree on left displays all the OTUs identified from sample, including related taxa of cultivated species with known hydrocarbonoclastic potential (shown in red). The diameter of nodes on the larger tree corresponds to qualitative number of OTU's in the respective taxa. Smaller trees represent significantly enriched taxa between paired treatments; ambient (400 ppm) or elevated CO_2 (750 ppm) at different timepoints corresponding to horizontal (brown) and vertical (green) axis. Sampling timepoints were t0 (the baseline in-situ community); t1 at day 4; t2 at day 15; t3 at day 22. Intensity of colour in smaller trees (brown or green) corresponds to difference in ratio of relative abundance of taxa in paired treatments therefore the greater the intensity of brown or green, the greeater the difference in ratio of taxa between treatments.

Table 5.2. Average relative abundance (%) ± standard error of fall bacterial OTU count between 400 ppm (ambient) and elevated CO2 (750 ppm) and enrichment/absence of crude oil (+/-) across timepoints (t0; baseline, t1; day 5, t2; day 15, and t3; day 22). Relative abundance (%) were rounded to nearest number and OTU's were grouped into respective phyla.

Timepoint	**T0 (Baseline)**	**T1**		**T2**				**T3**			
CO_2	**400 ppm**	**400 ppm**	**750 ppm**	**400 ppm**		**750 ppm**		**400 ppm**		**750 ppm**	
Oil enrichment / OTU	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
No. of OTU's	**377 ± 41**	**477 ± 56**	**606 ± 32**	**373 ± 45**	**732 ± 23**	**597 ± 96**	**603 ± 96**	**339 ± 57**	**663 ± 94**	**426 ± 167**	**483 ± 136**
Unclassified Bacteria	9 ± 2	3 ± 1	3 ± 1	2 ± 1	6 ± 0	3 ± 2	4 ± 1	5 ± 2	3 ± 1	2 ± <1	2 ± <1
Bacteroidetes (Phylum)											
Unclassified *Flavobacteriaceae*	4 ± 1	4 ± 1	3 ± 1	4 ± 1	4 ± 1	6 ± 2	1 ± <1	3 ± 1	2 ± 1	2 ± 2	4 ± 2
Polaribacter	3 ± 2	1 ± <1	1 ± <1	4 ± 1	1 ± <1	1 ± <1	<1 ± <1	1 ± <1	1 ± 1	1 ± 1	1 ± 1
Tenacibaculum	1 ± <1	1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	1 ± <1	1 ± <1	<1 ± <1	1 ± 1
Cyanobacteria (Phylum)											
Unclassified *GpIIa*	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1
Firmicutes (Phylum)											
Unclassified *Bacillaceae*	<1 ± <1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1	0 ± 0	0 ±0	0 ± 0	0 ± 0
Unclassified *Bacillales*	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	0 ± 0	<1 ± <1	3 ± 2	<1 ± <1	<1 ±<1	<1 ± <1	0 ± 0
Streptococcus	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	0 ± 0	1 ± 1	0 ± 0	<1 ± <1	<1 ±<1	0 ± 0	<1 ± <1
Veillonella	<1 ± <1	0 ± 0	<1 ± <1	0 ± 0	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Continued Table 5.2. part 1/4

Timepoint	**T0 (Baseline)**	**T1**		**T2**				**T3**			
CO_2	**400 ppm**	**400 ppm**	**750 ppm**	**400 ppm**		**750 ppm**		**400 ppm**		**750 ppm**	
Oil enrichment / **OTU**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
Fusobacteria (Phylum)											
Cetobacterium	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	<1 ±<1	2 ± 2	<1 ± <1
Planctomycetes (Phylum)											
Unclassified *Planctomycetaceae*	<1 ± <1	<1 ± <1	2 ± 1	<1 ± <1	<1 ± <1	<1 ± <1	3 ± 3	<1 ± <1	<1 ±<1	3 ± 3	1 ± <1
Proteobacteria (Phylum)											
Unclassified *Proteobacteria*	2 ± 1	2 ± <1	3 ± <1	2 ± <1	3 ± <1	2 ± <1	2 ± 1	2 ± <1	2 ± <1	1 ± <1	2 ± 1
Alphaproteobacteria (Class)											
Unclassified *Alphaproteobacteria*	2 ± 1	2 ± <1	3 ± 1	1 ± <1	3 ± 1	1 ± <1	3 ± 1	2 ± 1	2 ± 1	3 ± 1	1 ± <1
Unclassified *Rhizobiaceae*	0 ± 0	<1 ± <1	1 ± <1	0 ± 0	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	0 ± 0	1 ± 1	<1 ±<1
Gemmobacter	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	2 ± 1	<1 ±<1
Unclassified *Rhodobacteraceae*	7 ± 2	13 ± 4	9 ± 2	14 ± 2	10 ± 1	16 ± 3	6 ± 2	7 ± 1	13 ± 2	6 ± 2	12 ± 3
Shimia	<1 ± <1	<1 ± <1	<1 ± <1	0 ± 0	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	2 ± 1
Sulfitobacter	1 ± <1	1 ± <1	1 ± <1	2 ± <1	<1 ± <1	2 ± <1	<1 ± <1	<1 ± <1	1 ± <1	1 ± <1	2 ± <1
Unclassified *Reyranella*	0 ± 0	<1 ± <1	<1 ± <1	0 ± 0	0 ± 0	0 ± 0	1 ± 1	0 ± 0	0 ± 0	<1 ± <1	0 ± 0
Unclassified *Erythrobacteraceae*	1 ± <1	<1 ± <1	1 ± <1	0 ± 0	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1

Continued Table 5.2. part 2/4

Timepoint	**T0 (Baseline)**	**T1**		**T2**				**T3**			
CO_2	**400 ppm**	**400 ppm**	**750 ppm**	**400 ppm**		**750 ppm**		**400 ppm**		**750 ppm**	
Oil enrichment / **OTU**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
Unclassified *Rhodospirillaceae*	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1
Unclassified *Candidatus* Pelagibacter	3 ± 1	2 ± 1	3 ± 1	1 ± 1	3 ± 1	3 ± 2	1 ± 1	3 ± <1	2 ± 1	1 ± <1	1 ± 1
Erythrobacter	<1 ± <1	1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	1 ± <1	1 ± 1	0 ± 0
Betaproteobacteria (Class)											
Unclassified *Betaproteobacteria*	1 ± <1	1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	2 ± 1	1 ± <1	1 ± <1	1 ± 1	<1 ± <1
Gammaproteobacteria (Class)											
Aeromonas	0 ± 0	<1 ± <1	<1 ± <1	<1 ± <1	0 ± 0	<1 ± <1	1 ± 1	<1 ± <1	0 ± 0	1 ± 1	<1 ± <1
Unclassified *Alteromonadaceae*	4 ± 2	5 ± 1	1 ± <1	9 ± 3	1 ± 1	4 ± 2	1 ± <1	2 ± 2	2 ± 1	5 ± 3	2 ± 1
Marinobacter	2 ± 2	1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	2 ± 1	1 ± 1	3 ± 3
Paraglaciecola	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	1 ± <1
Salinimonas	<1 ± <1	<1 ± <1	<1 ± <1	0 ± 0	<1 ±<1	1 ± <1	<1 ± <1	<1 ± <1	<1 ±<1	<1 ± <1	<1 ±<1
Unclassified *Colwelliaceae*	2 ± 0	3 ± 1	3 ± 1	1 ± 1	6 ± 1	3 ± 0	3 ± 2	6 ± 1	2 ± <1	2 ± 1	4 ± 2

Continued table 5.2 part 3/4

Timepoint	**T0 (Baseline)**	**T1**		**T2**				**T3**			
CO_2	**400 ppm**	**400 ppm**	**750 ppm**	**400 ppm**		**750 ppm**		**400 ppm**		**750 ppm**	
Oil enrichment / **OTU**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
Unclassified *Alteromonadales*	2 ± 1	3 ± <1	2 ± <1	4 ± 1	2 ± 1	2 ± 1	1 ± 1	3 ± <1	3 ± 1	3 ± 2	2 ± 1
Colwellia	19 ± 5	19 ± 3	19 ± 3	24 ± 6	24 ± 2	16 ± 4	20 ± 9	20 ± 3	33 ± 5	18 ± 8	12 ± 6
Unclassified *Enterobacteriaceae*	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	2 ± 1	0 ± 0	<1 ±<1	3 ± 2	2 ± 2
Unclassified *Gammaproteobacteria*	9 ± 2	14 ± 2	12 ± 1	11 ± 2	16 ± 1	9 ± 2	13 ± 5	16 ± 1	8 ± 1	12 ± 3	18 ±4
Alcanivorax	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	2 ± 1	<1 ± <1	<1 ±<1	1 ± <1	<1 ± <1
Unclassified *Halomonadaceae*	0 ± 0	1 ± 1	1 ± 1	0 ± 0	<1 ± <1	4 ± 1	<1 ± <1	2 ± 2	<1 ±<1	<1 ± <1	7 ± 6
Halomonas	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	1 ± <1	<1 ±<1	<1 ± <1	1 ± 1
Marinomonas	1 ± <1	2 ± 1	1 ± 1	1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	1 ± 1	<1 ±<1	<1 ± <1	1 ± <1
Neptuniibacter	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	3 ± 3	<1 ±<1	0 ± 0	<1 ± <1
Unclassified *Oceanospirillaceae*	<1 ± <1	1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	2 ± 1	<1 ± <1	2 ± 2	1 ± 1	<1 ± <1	<1 ± <1
Oleispira	<1 ± <1	1 ± <1	1 ± <1	2 ± 2	<1 ± <1	0 ± 0	0 ± 0	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1
Pseudohongiella	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ±<1	<1 ± <1	1 ± 1
Unclassified *Oceanospirillales*	0 ± 0	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	0 ± 0	<1 ± <1	1 ± <1
Acinetobacter	0 ± 0	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	<1 ± <1	1 ± <1	0 ± 0	<1 ± <1	1 ± <1
Psychrobacter	2 ± 1	1 ± <1	5 ± 2	1 ± <1	1 ± <1	<1 ± <1	5 ± 3	1 ± 1	2 ± 2	6 ± 5	1 ± 1

Continued Table 5.2. part 4/4

Timepoint	**T0 (Baseline)**	**T1**		**T2**				**T3**			
CO_2	**400 ppm**	**400 ppm**	**750 ppm**	**400 ppm**		**750 ppm**		**400 ppm**		**750 ppm**	
Oil enrichment / **OTU**	**-**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
Pseudomonas	1 ± <1	1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	1 ± <1	<1 ± <1	1 ± <1
Aliivibrio	3 ±2	3 ± 1	2 ± 1	0 ± 0	3 ± <1	1 ± <1	<1 ± <1	4 ± 2	<1 ± <1	<1 ± <1	1 ± 1
Unclassified *Vibrionaceae*	2 ± 1	1 ± <1	1 ± <1	<1 ± <1	3 ± <1	<1 ± <1	<1 ± <1	3 ± 1	<1 ± <1	<1 ± <1	1 ± <1
Pseudoxanthomonas	0 ± 0	<1 ± <1	<1 ± <1	0 ± 0	0 ± 0	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Epsilonbacteria (Class)											
Arcobacter	<1 ± <1	1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	<1 ± <1	1 ± 1	2 ± 1
Tenericutes (Phylum)											
Unclassified *Mycoplasmataceae*	0 ± 0	<1 ± <1	<1 ± <1	0 ± 0	0 ± 0	0 ± 0	<1 ± <1	0 ± 0	0 ± 0	1 ± 1	0 ± 0

5.3.4. Carbonate chemistry of seawater in microcosms

Gas mixing for elevated CO_2 treatment was carried out continuously throughout experiment and monitored using CO_2 sensors. The achieved elevated pCO_2 of 735 ± 12 ppm (Figure 5.8) was lower than targeted 750 ppm. There is no significant difference between the slope for elevated CO_2 and ambient CO_2 ($p = 0.07$) but the elevation of pCO_2 levels at the y-intercept are significantly different ($p < 0.0001$).

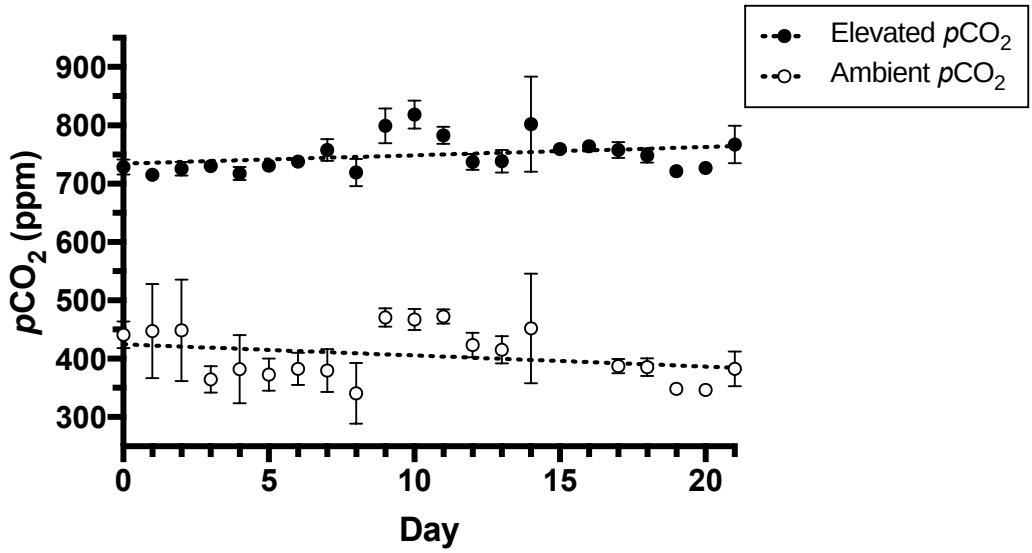


Figure 5.8. Daily average of CO_2 concentration (ppm) supplied to microcosms exposed to ambient and elevated pCO_2 conditions with linear regression (dotted lines) showing achieved level of ambient CO_2 (424 ± 18 ppm) and elevated pCO_2 (735 ± 12 ppm) concentration exposed to microcosms. Error bars shows standard deviation of pCO_2 per day.

CO_2 treatments were effective in altering seawater carbonate chemistry. Prior to oil enrichment, pH levels on day 4 were significantly different between CO_2 treatments ($p = 0.0003$). After exposure to crude oil, pH levels under ambient conditions remained significantly higher than those under elevated CO_2 on day 7 ($p < 0.0001$). Similarly, the pH of microcosms without oil-enrichment on day 7 were significantly different between ambient and elevated CO_2 ($p = 0.0054$). This same trend in pH levels remained the same for up to 18 days.

On day 14, pH levels of microcosms treated with oil under ambient conditions were significantly higher than those in microcosms under ambient conditions but with no oil ($p = 0.0042$) and under elevated CO_2 conditions treated with oil ($p < 0.0001$). pH levels of oil-enriched microcosms were significantly different between CO_2 treatments on day 18 ($p = 0.0087$). However, pH levels of non-oil treatments sampled at the same timepoint were not significantly different. On the last day of sampling (day 22), pH levels

were not significantly different between ambient CO_2-treated microcosms with/without oil and between elevated CO_2-treated microcosms with/without oil enrichment. However, pH levels were significantly different between ambient/without oil and elevated CO_2/oil-enriched microcosms ($p = 0.0069$), ambient/ oil-enriched and elevated CO_2/without oil ($p = 0.0110$), and ambient/oil-enriched and elevated CO_2/oil-enriched microcosms ($p = 0.0002$).

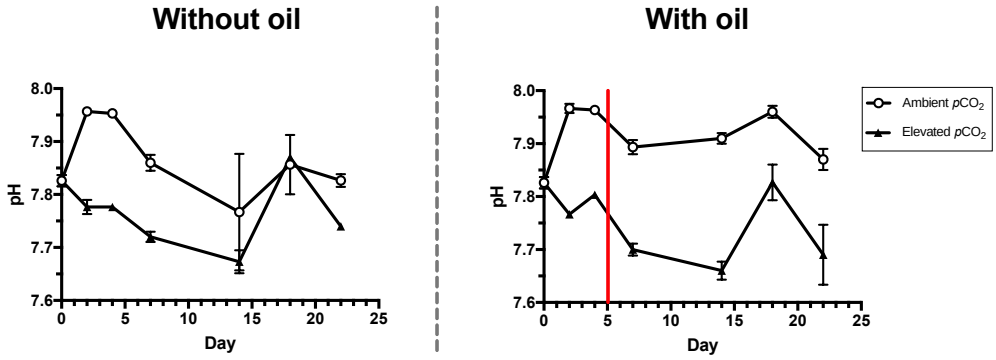


Figure 5.9. pH response of seawater in microcosms treated with ambient (open circles) and elevated CO_2 (closed triangle) in no-oil control (left) and with oil enrichment (right). Oil was added on day 5 (indicated by red lines).

The total dissolved inorganic carbon (C_T) that were titrated with acid and quantified by measuring total CO_2 and pH total scale (pH_T) measurements for each treatment were compared to baseline seawater (Appendix E). C_T of oil-enriched/elevated CO_2 treated microcosms were significantly higher than baseline seawater sample ($p = 0.0076$), ambient CO_2/without oil treated microcosms ($p = 0.0045$), ambient CO_2/oil-enriched microcosms ($p < 0.0001$), and elevated CO_2/without oil treated microcosms ($p = 0.0008$). Other seawater carbonate parameters were unsuccessfully calculated due to error in calibration of probe used for pH_T measurements and error in salinity measurements that led to high levels of calculated pCO_2 (Appendix E) when compared to monitored pCO_2 levels (Figure 5.8). Salinity was measured at the beginning of experiment, and not during the analysis for C_T, therefore evaporation during experiment that has occurred were not accounted for. As a result, carbonate chemistry parameters that were calculated such as total alkalinity (A_T), calcite saturation, bicarbonate, carbonate ions, and pCO_2 will not be assessed further.

5.3.5. Hydrocarbon profile

As similarly reported in the previous chapter (Chapter 4), the most abundant heteroatom classes that were found in crude oil extracts derived from the various microcosm treatments (ambient or elevated CO_2) were compared to those in oil extracts from acid-killed controls in order to identify any hydrocarbons that were biodegraded. Atmospheric pressure photoionisation (APPI) coupled with FT-ICR-MS analysis of hydrocarbon heteroatom (Appendix F), O1 heteroatom (Appendix G), and N1 heteroatom (Appendix H) were visualised using isoabundance-contoured plots. No differences were detected between the isoabundance plots for each heteroatom when compared to killed control.

Therefore, similar to findings with spring community from the same site in FSC (Chapter 4), biodegradation of crude oil was not detected by this method of ionisation of crude oil compounds. Although FT-ICR-MS allows for a wider analytical window of compounds, biomarkers for biodegradation of crude oil such as nC17, pristine, nC18, or phytane that are commonly detected using gas chromatography methods could not be detected here due to ionisation of compounds.

5.4. Discussion

5.4.1. Impacts of ocean acidification and oil enrichment on microalgal community

Taxonomic identification and enumeration of seawater samples collected in fall from FSC were dominated by unidentified athecate dinoflagellates (20 – 30 μm) is expected during fall in northeast Atlantic region (McQuatters-Gollop *et al.*, 2007). The scattered distribution of chl *a* of 0.1 mg/m^3 is typical of dinoflagellate population in the North Atlantic (Dale, Rey and Riddervold Heimdal, 1999). Dominance of smaller unidentified athecate are typical of nutrient-limited conditions as smaller species are more efficient at nutrient uptake (Sakshaug and Holm-Hansen, 1977; Smith and Kalff, 1982).

Successful acclimation to ambient (400 ppm) and elevated (750 ppm) CO_2 concentrations after 4 days were determined by significant difference in pH levels on day 4 ($p = 0.0003$) and higher concentration of chl *a*. Increase in chl *a* seen in microcosms treated with elevated CO_2 conditions concurs with mesocosm experiments (Liu *et al.*, 2017; Huang *et al.*, 2018). Despite reports of crude oil-tolerant dinoflagellate taxa such as *Scrippsiella sp*. that was identified in the baseline community, exposure to 1% crude oil were detrimental to the dinoflagellate-dominated microplankton community in microcosms (Almeda *et al.*, 2013).

No data was obtained on the percentage of heterotrophic or mixotrophic dinoflagellates compared to phototrophic species. Dinoflagellates are known to be able to switch from phototrophic to mixotrophic growth while some obligate heterotrophic dinoflagellates and ciliates may be parasitic or even prey on other microplankton in the microcosms (Mackey *et al.*, 2015). Consequently, bacterial and microalgal biomass and community composition plays an important role in availability of nutrients. Analysis of particulate organic carbon, N, and P concentration in microcosms at different timepoints would further inform the dynamic relationship between the microplankton community and their response to OA and crude oil enrichment. Some dinoflagellates release toxins under specific environmental conditions such as elevated CO_2, nutrient limitation that may affect microalgal and bacterioplankton community (Mackey *et al.*, 2015).

5.4.2. Impacts of ocean acidification on bacterial community and oil enrichment

No significant difference in alpha-diversity i.e. the abundance and evenness of taxa were detected, however 50% dissimilarity between baseline bacterial community of surface seawater collected from the FSC to the same community acclimated to ambient or elevated CO_2 concentration in microcosms after exposure to crude oil were observed (Figure 5.4). Species composition was not significantly altered from baseline community when tested with single or combined stressor of elevated CO_2 and oil enrichment.

Relative abundance of unclassified *Candidatus* Pelagibacter were higher under elevated CO_2 conditions and after oil-enrichment. Unclassified *Candidatus* Pelagibacter from the clade SAR11 have been found highly abundant in surface seawater, and is metabolically capable of oxidising a diverse range of single carbon compounds and methyl groups including dimethylsulfoniopropionate (DMSP) (Sun *et al.*, 2011). DMSP are osmolytes produced by microalgae and cyanobacteria that are precursors to dimethylsulfide that have a significant contribution to cloud condensation nuclei thus affecting climate, and the global sulphur cycle (Diaz, Visscher and Taylor, 1992; Malin, 1996). Single carbon compounds were likely in abundance as a by-product of exponentially growing microalgal community.

Elevated CO_2 conditions enriched *Psychrobacter* even after crude oil enrichment. No reports of hydrocarbon degradation was confirmed for *Psychrobacter*, but tolerance to cold, saline environments (Bozal *et al.*, 2003; Rodrigues *et al.*, 2009), metal contaminated environments (Abd-Elnaby *et al.*, 2016), hydrocarbon contaminated environments including dominance in marine oil snow (Suja, Summers and Gutierrez, 2017), and even in simulated Mars conditions (Smith *et al.*, 2009) suggests *Psychrobacter*

has high tolerance to a diverse range of stressful environments including oil enrichment under higher CO_2 conditions.

Similar to *Psychrobacter, Sulfitobacter* was also found enriched in elevated CO_2 conditions and oil-enriched treatments. Bacteria from the genus *Sulfitobacter* is an important microalgal associated bacteria that was previously isolated from diatom (Grossart *et al.*, 2005; Hong *et al.*, 2015) and coccolithophores (Green *et al.*, 2015). In addition to tolerating toxicity from diatom blooms, *Sulfitobacter* thrives when exposed to hydrocarbon pollution (Kostka *et al.*, 2011; Joye and Kleindienst, 2016), and under high pCO_2 conditions (Zhang *et al.*, 2012).

The *Alteromonadacea* family that includes *Marinobacter* were initially negatively impacted by elevated CO_2 conditions. After 22 days acclimated to elevated CO_2, relative abundance of unclassified *Alteromonadaceae* and *Marinobacter* were higher compared to ambient conditions. This was also seen after after 17 days of crude oil exposure under elevated CO_2 conditions suggesting persistence of taxa in low relative abundance and tolerance to combined stressors followed by opportunistic behaviour. Opportunistic behaviour was previously described in persistence of *Alteromonadaceae* in protist-bacteria association (Gong *et al.*, 2016), disturbances during mesocosm set-up, and in glucose amended mesocosm study (Allers *et al.*, 2007). *Marinobacter* have been reported to be associated with microalgae (Lupette *et al.*, 2016), capable of degrading hydrocarbon, and other hydrophobic organic carbon by producing biofilm to overcome low availability of hydrophobic organic compounds (Head, Jones and Röling, 2006; Grimaud *et al.*, 2012). Another well-known hydrocarbonoclastic bacteria, *Alcanivorax*, enriched after oil-enrichment in elevated CO_2 conditions. The relatively low mean percentage of *Alcanivorax* in all oil-enriched treatments (< 2%) compared to 80 – 90% as described by other studies was likely due to 1) co-limitation of other nutrients (nitrate and phosphate), 2) relatively lower temperature (10 ^{0}C instead of 20 ^{0}C), or 3) inhibition by other bacteria from the bacterial community associated to phytoplankton (Hara, Syutsubo and Harayama, 2003; Harayama, Kasai and Hara, 2004; Schneiker *et al.*, 2006).

Polaribacter was negatively affected by elevated CO_2 with and without the influence of oil enrichment. A highly adaptable organism such as *Polaribacter* was unexpectedly susceptible to elevation of CO_2 (750 ppm) and associated change in seawater carbonate chemistry. This was exacerbated by the addition of crude oil in elevated CO_2 conditions. *Polaribacter* have been described as a widely distributed in marine habitats including cold and temperate environments, in seawater and sediments, oligotrophic and copiotrophic nutrient regimens, coastal and open ocean environment,

including association to diatom bloom and phytoplankton bloom in the North Atlantic (Gómez-Pereira *et al.*, 2012; Teeling *et al.*, 2012; Xing *et al.*, 2015).

Colwellia has been the dominant bacterial taxa in ambient conditions with and without oil enrichment, and elevated CO_2/without oil enrichment. However, relative abundance of *Colwellia* decreased by 10 – 32% after 17 days of oil-enriched conditions under elevated CO_2 treatment at t3. *Colwellia* are psychrophilic heterotrophs capable of utilising a wide array of carbohydrates and amino acids (Methé *et al.*, 2005; Techtmann *et al.*, 2016). Besides tolerance in sea ice and polar sediments, *Colwellia* have been found associated to eukaryotes and was isolated from marine animals (Zhang *et al.*, 2008; Choi *et al.*, 2010; Kim *et al.*, 2013; Techtmann *et al.*, 2016). *Colwellia* is also capable of hydrocarbon degradation and was highly abundant during the Deepwater Horizon spill (Baelum *et al.*, 2012; Dubinsky *et al.*, 2013; Gutierrez *et al.*, 2013). Experiments involving bicarbonate assimilation in the dark have shown prevalence of *Colwellia* in high CO_2 environments that allows maintenance of cell activity and survival under oligotrophic Arctic conditions (Alonso-Sáez *et al.*, 2010). However, this is the first study that shows *Colwellia* negatively affected by oil enrichment and elevated CO_2 concentrations.

This shows that although most taxa were resilient to ocean acidification with or without the presence of crude oil like *Colwellia*, some taxa were susceptible to higher CO_2 and crude oil such as *Polaribacter* Additional stressors that were not accounted for includes impact of grazing community on heterotrophic bacteria abundance. Bacterial count and microplankton count were not carried out to identify dominating microplankton species throughout experiment, and relationship of counts between these groups. Grazing resistant bacteria may have mechanisms such as morphological, chemical, or behavioural defences against grazers (Jurgens and Gude, 1994). This may indirectly influence the response of bacterioplankton community favouring grazing resistant bacteria when exposed to elevated CO_2 and/or oil enriched treatments. Furthermore, selection and dominance of bacterial taxa is highly likely due to co-limiting nutrient factors in addition to CO_2 and oil stressors. Therefore, nutrient analysis corresponding to community sampling timepoints would be beneficial in future studies.

5.4.3. Carbonate chemistry of subsurface seawater from FSC in microcosms

Microcosms were successfully bubbled with elevated pCO_2 (735 ± 12 ppm; Figure 5.8) close to targeted levels (750 ppm) proposed in the Guide to Best Practices for Ocean Acidification Research and Data Processing (Riebesell *et al.*, 2011). pH levels

reduced by 0.2 units even after oil enrichment (Figure 5.9) as expected for targeted level of pCO_2 in seawater (Riebesell *et al.*, 2011) while C_T of elevated CO_2 oil-enriched microcosms were significantly higher compared to all other treatments is expected of due to enrichment of inorganic carbon from CO_2 bubbling and crude oil enrichment.

5.4.4. Biodegradation of crude oil in oil-enriched microcosms

Isoabundance-contoured plots of hydrocarbon, O1, and N1 heteroatoms from FT-ICR-MS analysis allows a relatively larger analytical window compared to gas chromatography approaches. Isoabundance-contoured plots clearly show no degradation of crude oil occur in both the ambient and elevated CO_2 treated microcosms during 17 days of crude oil exposure in microcosms. Although relative abundance of hydrocarbon degrading bacteria such as *Alcanivorax, Psychrobacter, Pseudomonas* and *Marinobacter*, were enriched, the mean percentage of the potentially hydrocarbonoclastic bacteria were low (< 2% of total bacterial community). *Colwellia* and unclassified *Gammaproteobacteria* were highly abundant taxa in the baseline community remained highly abundant even after different CO_2 regimens were applied to the microcosms. An apparent lack of hydrocarbon biodegradation suggests that persistence of *Colwellia* and unclassified *Gammaproteobacteria* caused competition for common resources for hydrocarbonoclastic bacteria or sub-inhibitory levels of antimicrobials acting on the hydrocarbonoclastic bacteria (Hibbing *et al.*, 2010). Furthermore, temperature at which microcosms were maintained (10 ^{0}C) could have also contributed to the lack of hydrocarbon biodegradation after 17 days of exposure to crude oil. Slower evaporation of toxic volatile compounds of crude oil at 10 ^{0}C were found to inhibit microbial degradation (Atlas, 1975).

5.5. Conclusion

The microalgal community were positively affected by CO_2 enrichment in microcosms and was susceptible to crude oil enrichment (1% v/v) in both ambient and elevated CO_2 treatments. The success of bacterial taxa in oil-enriched ocean acidification conditions were attributed to persistence and tolerance to crude oil toxicity as shown by *Colwellia, Psychrobacter* and *Sulfitobacter* in addition to opportunistic behaviour displayed by *Marinobacter* and unclassified *Alteromonadaceae.* Despite the dynamic shifts in relative abundance of bacterial taxa, the impact of ocean acidification on the biodegradation of crude oil was not apparent after 17 days of exposure to crude oil. The dominating taxa that were tolerant to combined stressors introduces competition for

resources, and potentially inhibition of hydrocarbonoclastic bacteria by antimicrobials. Low temperature of the subarctic region could also hinder biodegradation of crude oil, thus recovery of subarctic oceanic waters from oil pollution.

Chapter 6: Synthesis and Future Work

The objective of this PhD project was to determine the response of open ocean microbial community, specifically microalgae and their associated bacterial community to ocean acidification and potential crude oil pollution. In order to understand the microbiological response, chlorophyll *a* (chl *a*) concentration were used as a proxy to determine microalgal abundance and response to stressors, and taxonomic identity and relative abundance of bacterial community were assessed to identify resilience or susceptibility of bacterial taxa to crude oil exposure under ocean acidification stress. Seawater carbonate chemistry and hydrocarbon profiling describe changes in seawater chemistry impacting microbial community in the microcosms. This chapter summarize the work carried out within this PhD project followed by conclusions and proposed future work.

6.1. Summary

6.1.1. Chapter 3: Impact of ocean acidification and crude oil pollution on Emiliania huxleyi and its microbiota.

The aim of chapter 3 was to investigate the response of *E. huxleyi* and its bacterial consortia acclimated to 750 ppm of CO_2 to crude oil pollution (1% v/v). Findings from this chapter can be summarised by:

1) *E. huxleyi* chl *a* concentration was positively affected by elevated CO_2 conditions.
2) *E. huxleyi* was susceptible to crude oil enrichment especially in elevated CO_2 conditions as chl *a* concentration was undetected after 10 days of crude oil exposure.
3) Decrease in relative abundance of host-associated *Marinobacter* and *Methylobacterium* potentially affected survival of host to crude oil pollution under elevated CO_2 conditions.
4) No bacterial taxa associated to *E. huxleyi* were enriched by ocean acidification conditions.
5) Other than *Sphingomonas* and *Methylobacterium*, bacterial community associated to *E. huxleyi* were resilient to crude oil pollution in ocean acidification conditions.

6) Biodegradation of aliphatic hydrocarbon compounds pristane and phytane were not affected by ocean acidification conditions in nutrient (f/2) enriched seawater.

6.1.2. Chapter 4: Response of spring northeast Atlantic bacterioplankton community to crude oil under elevated CO_2.

The aim of Chapter 4 was to determine the response of natural microbial community sampled during spring (late May) to ocean acidification and crude oil pollution. Findings from this chapter includes:

1) Microflagellate dominated community with high abundance of diatoms, and coccolithophores were found extensively in FSC in low chl *a* concentration.
2) Chl *a* concentration was positively affected by elevated CO_2 conditions and negatively affected by crude oil enrichment.
3) Chl *a* concentration recovered from toxicity of crude oil 13 days after oil exposure in both CO_2 conditions.
4) *Sulfitobacter* and *Acinetobacter* were positively influenced by ocean acidification conditions and oil enrichment.
5) Members of *Gammaproteobacteria, Halomonadaceae,* and *Rhodobacteriaceae* were positively influenced by ocean acidification conditions.
6) *Polaribacter* and *Aliivibrio* negatively affected by ocean acidification and oil enrichment.
7) *Colwellia, Polaribacter, Marinobacter*, unclassified *Flavobacteriaceae,* and unclassified bacteria were negatively affected by OA without oil enrichment.
8) *Colwellia* and members of *Alteromonadaceae* were susceptible to ocean acidification conditions but were enriched under elevated CO_2 conditions after crude oil enrichment.
9) Unclassified *GpIIa* cyanobacteria was positively affected by elevated CO_2 9 days after oil enrichment.
10) *Marinomonas* was negatively affected by elevated CO_2 conditions with and without oil enrichment.
11) Biodegradation of crude oil was not detected after 16 days of oil exposure.

6.1.3. Chapter 5: Response of fall northeast Atlantic bacterioplankton community to crude oil under elevated CO2.

The aim of Chapter 5 was to determine the natural microbial community sampled during fall in response to ocean acidification and crude oil pollution as a seasonal comparison to spring community response to the same stressors. Findings from this chapter includes:

1) Dinoflagellate dominated microalgal community responded positively to elevated CO_2 (750 ppm) concentrations.
2) Crude oil pollution was detrimental to microalgal community under ambient and elevated CO_2 conditions.
3) *Psychrobacter, Sulfitobacteria* and *Candidatus* Pelagibacter were positively affected by elevated CO_2 with and without oil-enrichment.
4) *Polaribacter*, members of *Alteromonadales*, *Alteromonadacaeae*, and *Flavobacteria* were negatively affected by elevated CO_2.
5) Despite negatively affected by elevated CO_2, *Colwellia* was prevalent when treated with crude oil under elevated CO_2.
6) *Marinobacter* negatively affected by high CO_2 at t1, and t2, but was positively affected at t3 without oil enrichment.
7) Biodegradation of crude oil compounds was not detected up to 17 days of oil exposure.

6.2. Conclusion

The fundamental ecological importance of microalgal population as the base of the food chain for organisms of higher trophic levels is especially important in the open ocean. The relationship between microalgae and heterotrophic bacteria shifts between mutualism, commensalism and parasitism depending on factors like nutrient availability, N:P ratio, and light intensity (Fuentes et al., 2016). The factors that alters microbial interaction can come from change in seawater chemistry by indirect effects of projected OA conditions on a global scale, or from oil enrichment in the event of an oil spill on a localised scale. Therefore, the fate of an ecosystem rests on bacterial interaction with microalgae for primary production, and bacterial mediated degradation of crude oil for recovery of an ecosystem from oil pollution.

In chapter 3, it was observed that *E. huxleyi*, a coccolithophore that harbours *Marinobacter* (a genus of hydrocarbonoclastic bacteria) together with other dominant hydrocarbon-degrading representatives, such as *Methylobacterium* and *Sphingomonas* (Green, 2006; Kertesz and Kawasaki, 2010) responded differently under OA conditions. The host *E. huxleyi* increased chl *a* concentration when treated with elevated CO_2 concentrations but showed higher susceptibility to crude oil. Shift in *E. huxleyi* associated bacterial community did not affect hydrocarbon degradation in OA conditions. Findings from this chapter are applicable in the Faroe Shetland Channel (FSC) in particular, since annual spring blooms recorded in Foinaven oil field during May were dominated by coccolithophores (Debes, 2000). The extent and timing of microalgal blooms dictate survival and reproduction of primary consumers such as copepods (Gaard, 2000), krill (Debes, Gallego and Magen, 2007), and deep-sea sponges (Spetland *et al.*, 2007; JNCC, 2018). Therefore, the future for microalgal community in FSC looks promising as OA promises higher chl *a* concentration thus, increasing photosynthesis rates. In the untimely event of an oil spill from one of the many oil fields in FSC during a coccolithophore bloom, future ocean conditions will unlikely affect the hydrocarbon biodegradation process carried out by the microbial community, despite shifts in the structure of the hydrocarbon-degrading community. However, laboratory cultures of *E. huxleyi* and associated bacteria lack complexity of interaction with other microalgal population and their associated bacterial community. In addition, *E. huxleyi* were maintained under f/2 nutrient amended seawater.

Therefore, this hypothesis was tested on surface seawater samples along with its natural microplankton and associated bacterioplankton consortia for a closer to real world scenario. Samples were collected from FSC during spring and maintained in microcosms under elevated CO_2 concentration and oil enrichment, without nutrient amendment. Similar to laboratory cultures, increasing levels of CO_2 in the atmosphere was found to have a fertilising effect on microalgal abundance, even aiding the recovery of spring microalgal community after 13 days of oil exposure. Although this may benefit organisms of higher trophic levels, high CO_2 adversely affected the bacterial community. Key hydrocarbon degraders such as *Marinobacter*, *Alcanivorax*, and *Oleispira* were detected in the microcosms in low relative abundance. Biodegradation of crude oil were impeded in both ambient and elevated CO_2 conditions up to 16 days after oil enrichment.

The response of microalgal community collected during fall showed similar response of increased chl *a* concentration from OA conditions. However, fall microalgal population did not recover after crude oil enrichment. Resilience of dominating taxa under OA and oil exposure hindered hydrocarbonoclastic bacteria from dominating the bacterial community. Simulation of oil spill, as well as case studies from the Deepwater Horizon spill have shown blooms of hydrocarbonoclastic bacteria to occur within 8 to 14 days of oil enrichment under nutrient replete conditions (Kostka *et al.*, 2011; Lu *et al.*, 2012; Joye and Kleindienst, 2016; Kleindienst *et al.*, 2016).

It appeared that highly abundant taxa from subarctic FSC like *Colwellia*, members of *Gammaproteobacteria, Rhodobacteriacea and Halomonadaceae* were resilient to the combined impact of ocean acidification and oil pollution whereas taxa with lower relative abundance such as *Polaribacter* were more susceptible to both stressors. *Sulfitobacter* and *Psychrobacte*r were positively affected by both stressors but remained in relatively low abundance compared to *Colwellia, Gammaproteobacteria, Rhodobacteriacea and Halomonadaceae*. The resilience of these highly abundant taxa has outcompeted or possibly inhibited specialist bacteria such as the hydrocarbonoclastic *Marinobacter, Alcanivorax*, and *Oleispira* even after oil-enrichment. Although biodegradation of crude oil was not observed up to 16 days after oil-enrichment in both spring and fall experiments, this is likely due to quantitative resolution of FT-ICR-MS that was not capable of detecting degradation of compounds after 16 days of oil enrichment. Therefore, the findings of this thesis portend persistence of highly abundant bacteria from subarctic region that were resilient to change in seawater chemistry, be it from accelerated global changes like ocean acidification, or immediate localised changes like marine pollution potentially impedes functional roles of specialist bacteria. Questions remain regarding 1) what the functional role of these resilient generalist bacteria have within the subarctic ecosystem; and 2) how they interact with bacteria of lower relative abundance such as specialist bacteria under combined OA and oil polluted conditions.

6.3. Future work

The taxonomic data from high-throughput sequencing of 16S rRNA presented in this thesis identified resilient taxa and key players affected by ocean

acidification and oil enrichment. Now that we have identified "who is there?", it would be logical to question "what are they doing?". Often in nature, keystone species that maintains integrity and structure of the community has a large impact disproportionate to its abundance (Dodds *et al.*, 2010). Dynamic shifts of bacterial taxa in this study was mostly of the taxa with smaller relative abundance (1 – 5%). While the dominant taxa have shown change in relative abundance, the changes occurring in the lower relative abundance taxa may have long-term implications on the community. It is hypothesised that co-limitation of nutrients and possible inhibition by dominant taxa were the cause for low relative abundance of hydrocarbon degrading taxa. In order to test this hypothesis, nutrient amended treatments could be done in addition to the original treatments to determine if seawater from FSC lacks macro- and/or micronutrients necessary for the biodegradation of crude oil. Furthermore, identifying potential genes for antimicrobials and other important genes within the community affected by changes in the microbial community could be done using the metagenomics approach. In addition, assessment of PIC/POC, N, and P concetration that corresponds to sampling time of microalgal and bacterial community throughout experiment would be vital in understanding dynamic interaction of bacterioplankton with eukaryotic microplankton over time.

Metagenomics is a useful tool that uses a heterogenous mix of genome fragments from species, strains and subpopulations to be catalogued for understanding of community dynamics, selection processes, and species interaction (Tringe and Rubin, 2005). Metagenomics also allows genome assembly and an insight to the role of host-associated bacteria that is previously uncultured i.e unclassified taxa (Venter *et al.*, 2004; Tringe and Rubin, 2005). Although genome assembly is an alluring idea with some previous historical success, not all genomes are as easily assembled, and errors come from the limitation of sequencing technology (Baker, 2012). Therefore it would be interesting to use metagenomics to identify culturing conditions for the previously uncultured bacteria (Garza and Dutilh, 2015; Lagier *et al.*, 2016).

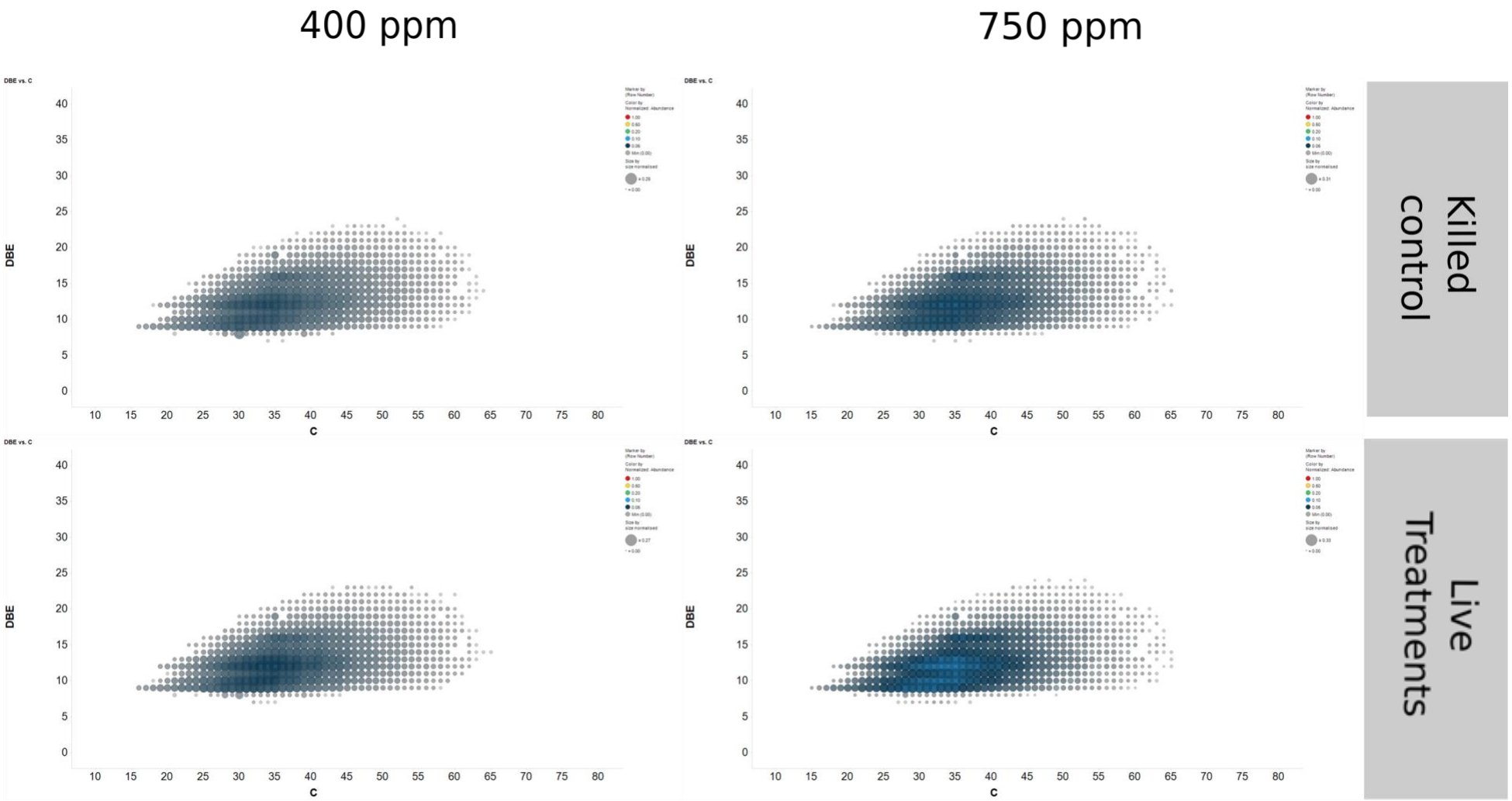
In addition to in-depth molecular analysis, increasing the number of CO_2 treatments would be beneficial in investigating the effects of ocean acidification on microalgal-bacteria relationship and response to oil pollution. Expanding the key atmospheric CO_2 concentrations from ambient (400 ppm) and moderate future

projection (750 ppm) to include pre-glacial (280 ppm), and higher future projection (1000 ppm) depends on the number of treatments possible in experimental setup as recommended by Barry *et al.* (2010). This enables identification of tipping point of biological responses, and provides greater power in characterising the effects of ocean acidification on microbial community and its functional role in the upper ocean (Barry *et al.*, 2010). Furthermore, increasing exposure time to crude oil by lengthening the number of days for the experiment could be useful in determining when biodegradation occurs in natural community of bacteria. Investigations into the algal-bacterial response to ocean perturbations, especially to anthropogenic stressors, is becoming ever more important to better understand community changes and what impacts this might have to local and further afield marine ecosystems, as well as to global planetary processes.

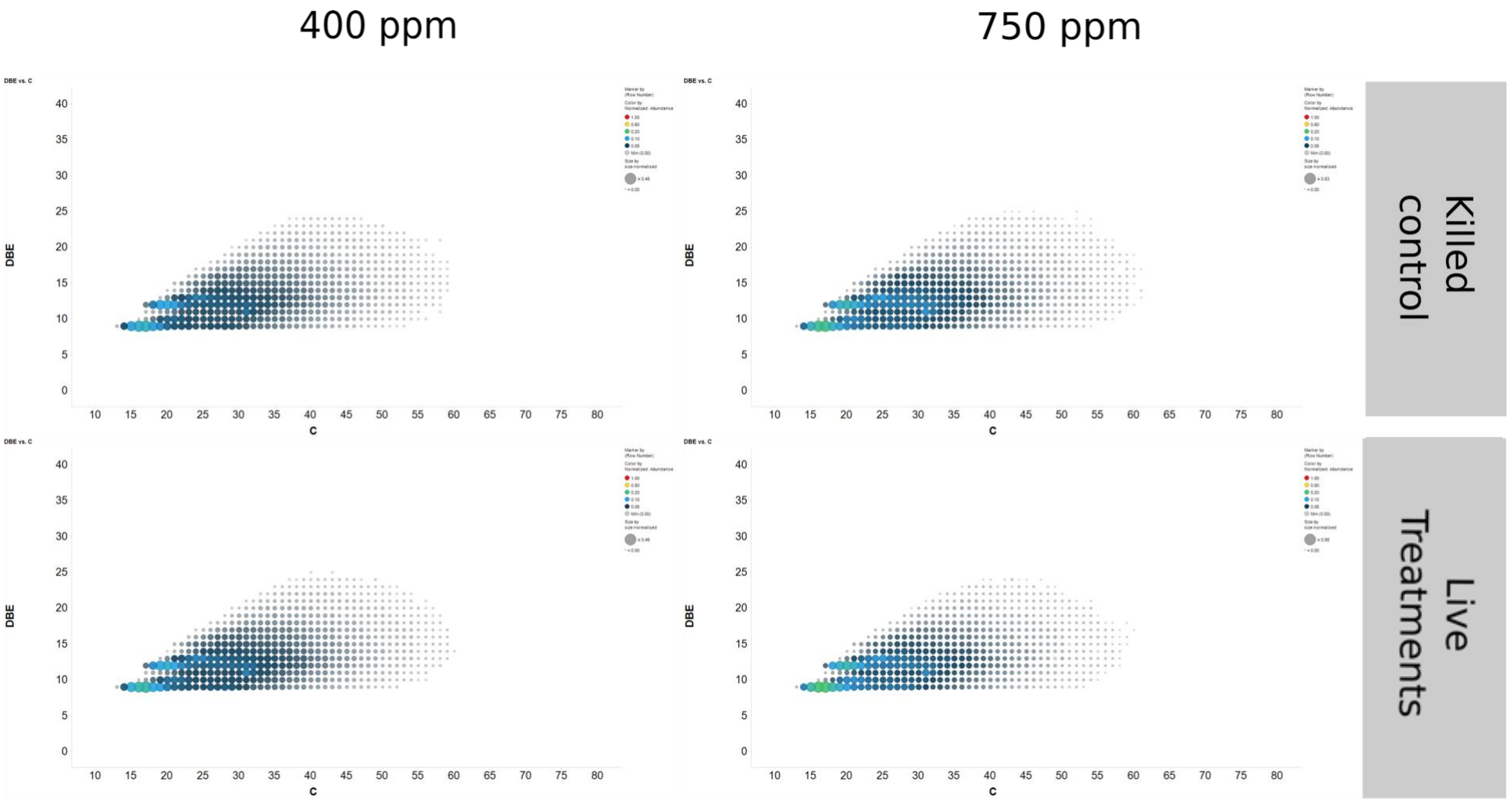
Appendices

Appendix A. Carbonate chemistry parameters (± standard error of means) seawater collected from FSC (baseline) during spring in microcosms exposed to ambient (400 ppm) or elevated (750 ppm) CO_2 concentration in the absence (- oil) or presence (+ oil) of oil. Total alkalinity (A_T), dissolved inorganic carbon (C_T), pH total scale (pH_T), pCO_2 in the seawater (µatm) and saturation of calcite ($\Omega_{Calcite}$).

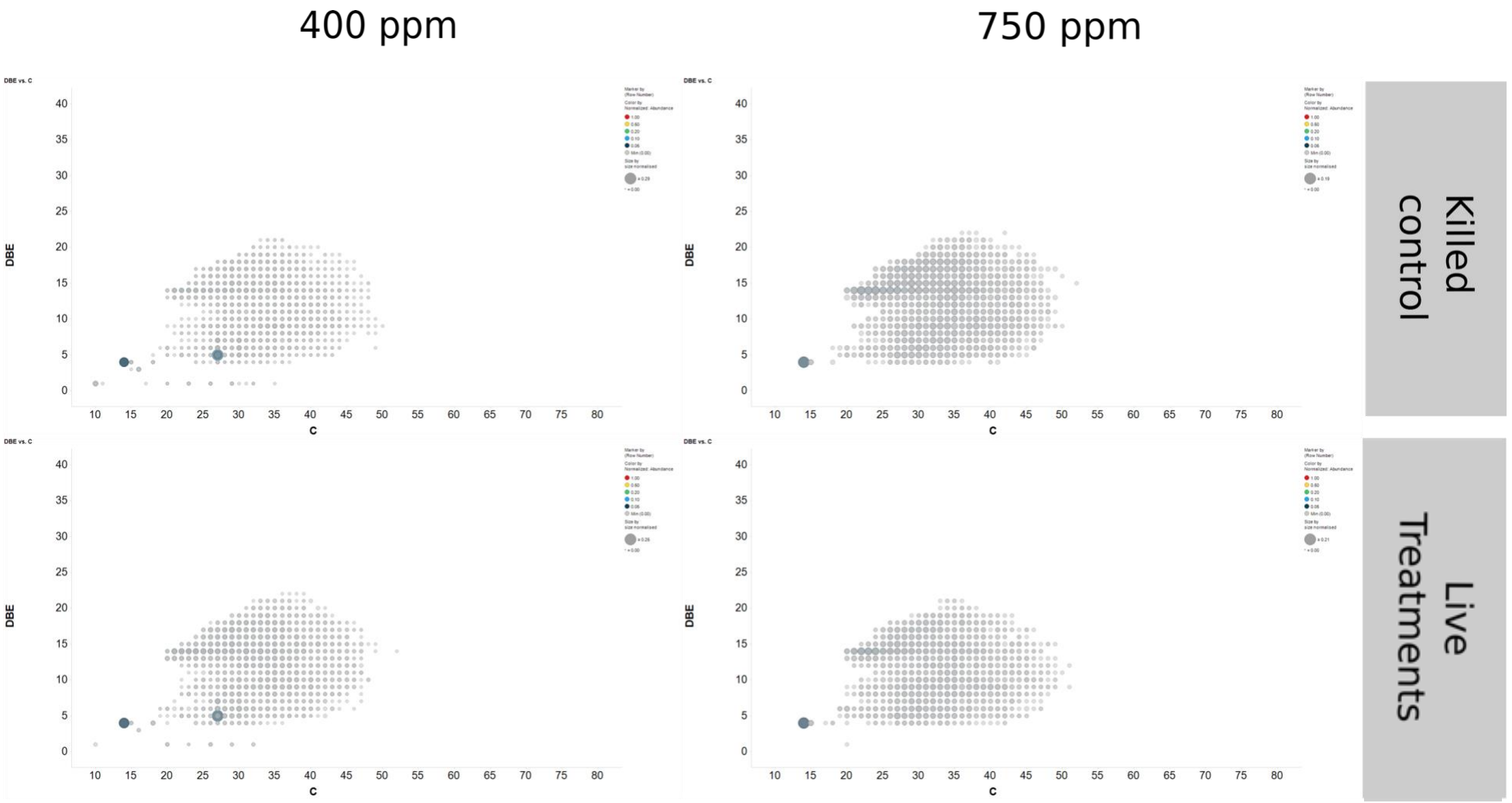
	A_T $\mu mol\ kg^{-1}$	C_T $\mu mol\ kg^{-1}$	pH_T	$\Omega_{calcite}$	HCO_3^{-1} $\mu mol\ kg^{-1}$	CO_3^{2-} $\mu mol\ kg^{-1}$	pCO_2 μatm
Baseline (Spring)	2320.42 ± 6.96	2112.29 ± 1.92	7.87 ± 0.01	3.75 ± 0.09	1937.92 ± 2.12	155.97 ± 3.56	650.92 ± 15.72
400 ppm - oil	2282.37 ± 16.37	2147.5 ± 17.7	7.71 ± 0.05	2.69 ± 0.26	2007.47 ± 22.27	111.87 ± 10.79	996.15 ± 122.09
400 ppm + oil	2248.38 ± 39.78	2138.8 ± 36.4	7.64 ± 0.04	2.33 ± 0.21	2009.65 ± 34.59	96.87 ± 8.61	1143.51 ± 106.09
750 ppm - oil	2285.95 ± 23.72	2204.12 ± 24.85	7.57 ± 0.03	2.01 ± 0.14	2080.45 ± 24.57	83.71 ± 6.01	1413.78 ± 124.09
750 ppm + oil	2250.70 ± 26.15	2210.06 ± 27.02	7.45 ± 0.02	1.53 ± 0.06	2093.62 ± 25.66	63.83 ± 2.61	1861.02 ± 88.01



Appendix B. Isoabundance-contoured plots of double bond equivalents (DBE) versus carbon number for N_1 heteroatom class between samples inoculated with spring seawater FSC microbial community exposed to ambient (400 ppm) and elevated (750 ppm) CO_2 treatments compared with non-biodegraded killed controls.



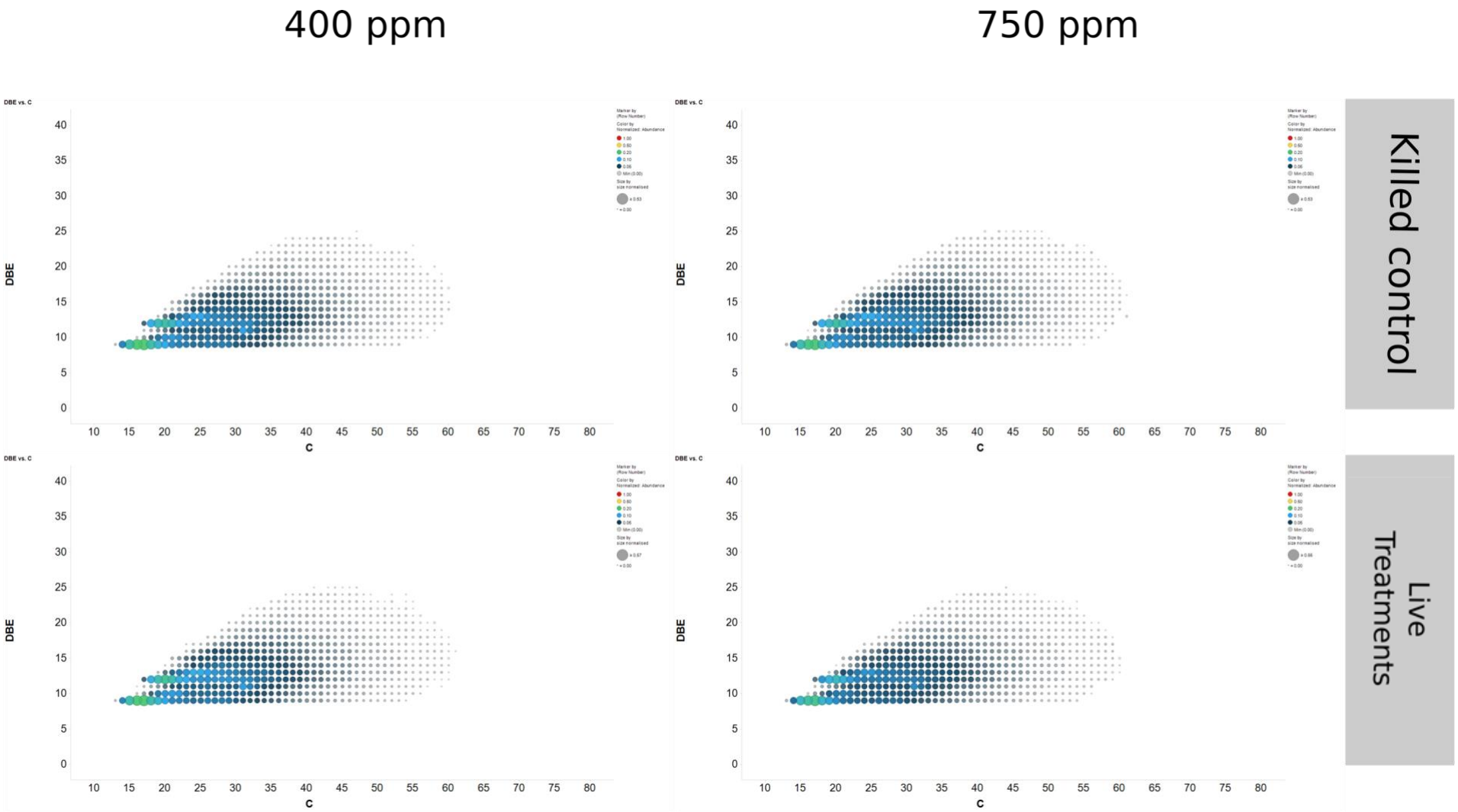
Appendix C. Isoabundance-contoured plots of double bond equivalents (DBE) versus carbon number for hydrocarbon heteroatom class between samples inoculated with spring seawater FSC microbial community when exposed to ambient (400 ppm) and elevated (750 ppm) CO_2 treatments compared with non-biodegraded killed controls.



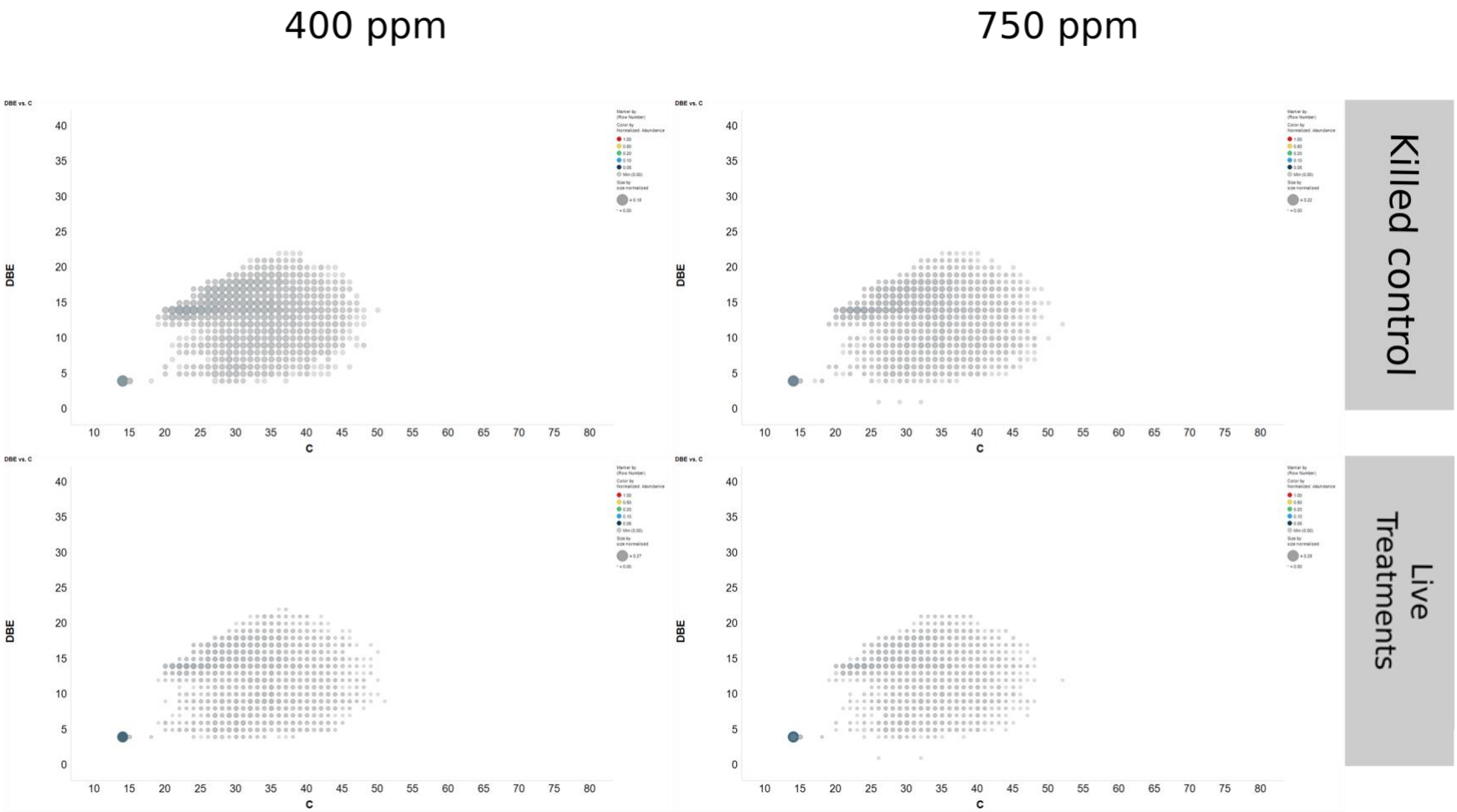
Appendix D. Isoabundance-contoured plots of double bond equivalents (DBE) versus carbon number for O_1 heteroatom class between samples inoculated with spring seawater FSC microbial community exposed to ambient (400 ppm) and elevated (750 ppm) CO_2 treatments compared with non-biodegraded killed control.

Appendix E. Carbonate chemistry parameters (± standard error of means) for seawater collected from FSC (baseline) during fall in microcosms exposed to ambient (400 ppm) or elevated (750 ppm) CO_2 concentration in the absence (- oil) or presence (+ oil) of oil. Total alkalinity (A_T), dissolved inorganic carbon (C_T), pH total scale (pH_T), pCO_2 in the seawater (µatm) and saturation of calcite ($\Omega_{Calcite}$).

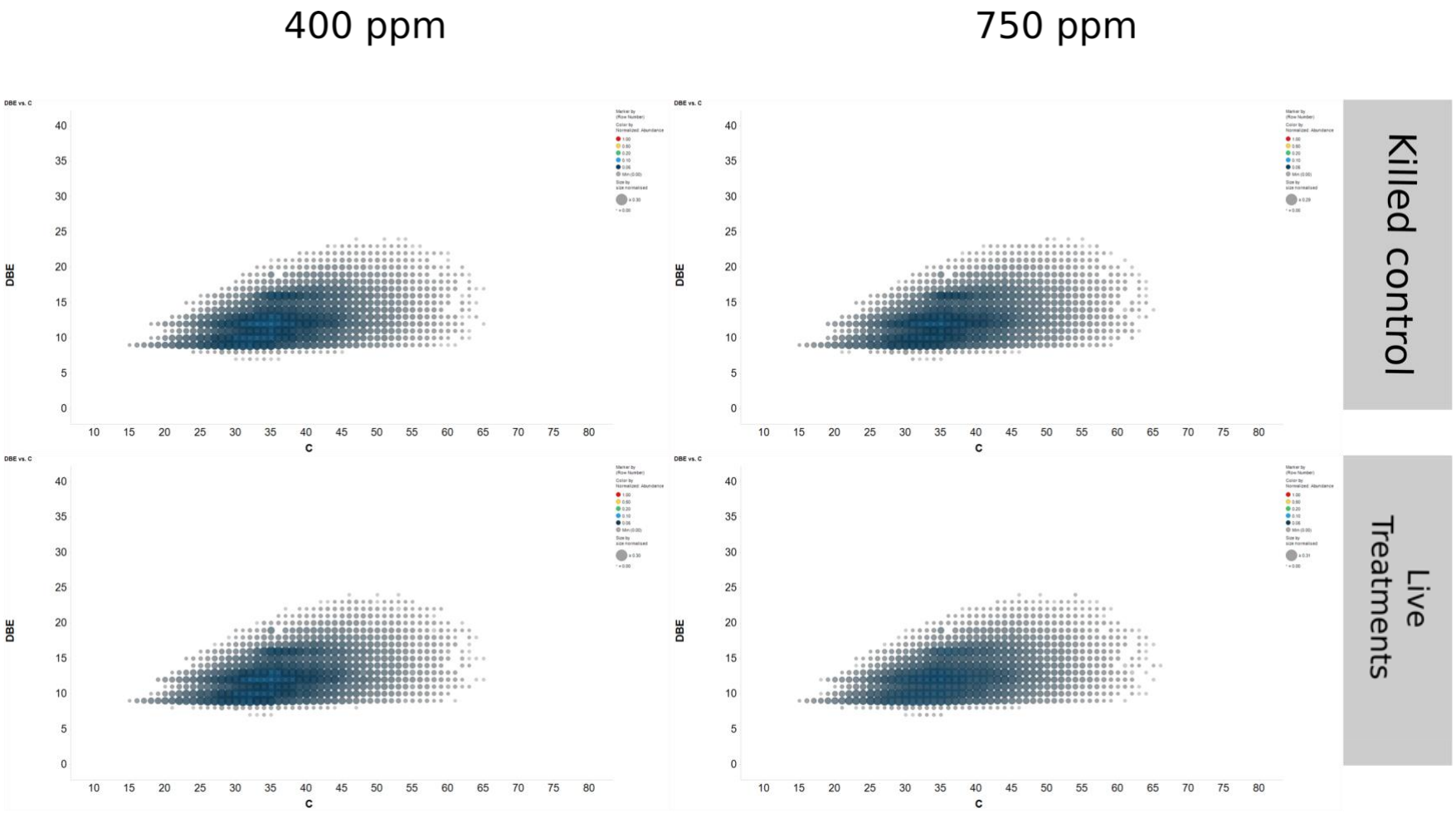
	A_T	C_T	pH_T	$\Omega_{calcite}$	HCO_3^{-1}	CO_3^{2-}	pCO_2
	$\mu mol\ kg^{-1}$	$\mu mol\ kg^{-1}$			$\mu mol\ kg^{-1}$	$\mu mol\ kg^{-1}$	μatm
Baseline (Fall)	2277.34 ± 5.57	2095.13 ± 0.56	7.83 ± 0.01	3.36 ± 0.08	1935.52 ± 2.45	139.23 ± 3.30	718.80 ± 19.06
400 ppm - oil	2215.47 ± 29.05	2090.51 ± 20.43	7.69 ± 0.03	2.52 ± 0.17	1958.12 ± 16.77	104.38 ± 7.14	987.9 ± 58.26
400 ppm + oil	2162.31 ± 26.44	2028.57 ± 26.26	7.72 ± 0.00	2.60 ± 0.02	1895.72 ± 24.92	107.57 ± 0.89	891.43 ± 16.26
750 ppm - oil	2151.51 ± 88.58	2075.99 ± 62.66	7.55 ± 0.10	1.90 ± 0.42	1956.94 ± 56.35	78.69 ± 17.29	1423.25 ± 279.28
750 ppm + oil	2262.80 ± 2.73	2187.10 ± 7.09	7.56 ± 0.01	1.93 ± 0.05	2066.69 ± 7.77	79.90 ± 2.06	1428.79 ± 48.43



Appendix F. Isoabundance-contoured plots of double bond equivalents (DBE) versus carbon number for hydrocarbon heteroatom class in ambient (400 ppm) or elevated (750 ppm) CO_2 treatments exposed to acid-killed control or live inoculants of microbial community from FSC.



Appendix G. Isoabundance-contoured plots of double bond equivalents (DBE) versus carbon number for O_1 heteroatom class in ambient (400 ppm) or elevated (750 ppm) CO_2 treatments exposed to acid-killed control or live inoculants of microbial community from FSC.



Appendix H. Isoabundance-contoured plots of double bond equivalents (DBE) versus carbon number for N_1 heteroatom class in ambient (400 ppm) or elevated (750 ppm) CO_2 treatments exposed to acid-killed control or live inoculants of microbial community from FSC.

References

Abd-Elnaby, H. M. *et al.* (2016) 'Characterization and bioremediation potential of marine Psychrobacter species', *The Egyptian Journal of Aquatic Research*. Elsevier, 42(2), pp. 193–203. doi: 10.1016/J.EJAR.2016.04.003.

Acinas, S. G. *et al.* (2005) 'PCR-Induced Sequence Artifacts and Bias: Insights from Comparison of Two 16S rRNA Clone Libraries Constructed from the Same Sample', *Applied and Environmental Microbiology*, 71(12), pp. 8966–8969. doi: 10.1128/AEM.71.12.8966-8969.2005.

Adhikari, P. L., Maiti, K. and Overton, E. B. (2015) 'Vertical fluxes of polycyclic aromatic hydrocarbons in the northern Gulf of Mexico'. doi: 10.1016/j.marchem.2014.11.001.

Afi, L. *et al.* (1996) *Bacterial degradation of green microalgae: incubation of Chlorella emersonii and Chlorella vulgaris with Pseudomonas oleovorans and Flavobacterium aquatUe, Org. Geochem*. Available at: https://ac-els-cdn-com.ezproxy1.hw.ac.uk/S0146638096001131/1-s2.0-S0146638096001131-main.pdf?_tid=9748c54f-f7be-4998-972c-a1e9a29d0932&acdnat=1533306997_68a7e22ad3b849d16221ee1400c6a374 (Accessed: 3 August 2018).

Agostini, P., Riebesell, U. and Gattuso, J.-P. (2011) *Directorate-General for Research and Innovation Directorate I – Environment Unit I.4 – Climate change and natural hazards*. Edited by U. Riebesell et al. Hazards, Directorate-General for Research and Innovation Directorate I – Environment Unit I.4 – Climate change and natural. Available at: http://www.davidluquet.com/ (Accessed: 3 January 2018).

Al-Hasan, R. H. *et al.* (2001) 'Hydrocarbon accumulation by picocyanobacteria from the Arabian Gulf.', *Journal of applied microbiology*, 91(3), pp. 533–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11556921 (Accessed: 9 May 2018).

Alldredge, A. L. and Cohen, Y. (1987) 'Can Microscale Chemical Patches Persist in the Sea? Microelectrode Study of Marine Snow, Fecal Pellets', *Science*, 235(4789), pp. 689–691. doi: 10.1126/science.235.4789.689.

Allers, E. *et al.* (2007) ‘Response of Alteromonadaceae and Rhodobacteriaceae to glucose and phosphorus manipulation in marine mesocosms’, *Environmental Microbiology*. Wiley/Blackwell (10.1111), 9(10), pp. 2417–2429. doi: 10.1111/j.1462-2920.2007.01360.x.

Almeda, R. *et al.* (2013) ‘Effects of crude oil exposure on bioaccumulation of polycyclic aromatic hydrocarbons and survival of adult and larval stages of gelatinous zooplankton.’, *PloS one*. Public Library of Science, 8(10), p. e74476. doi: 10.1371/journal.pone.0074476.

Alonso-Sáez, L. *et al.* (2010) ‘High bicarbonate assimilation in the dark by Arctic bacteria’, *The ISME Journal*. Nature Publishing Group, 4(12), pp. 1581–1590. doi: 10.1038/ismej.2010.69.

Amin, S. A. *et al.* (2009) ‘Photolysis of iron-siderophore chelates promotes bacterial-algal mutualism.’, *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 106(40), pp. 17071–6. doi: 10.1073/pnas.0905512106.

Amin, S. A. *et al.* (2012) ‘Iron transport in the genus Marinobacter’, *BioMetals*, 25(1), pp. 135–147. doi: 10.1007/s10534-011-9491-9.

Amin, S. A. *et al.* (2015) ‘Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria’, *Nature*. Nature Publishing Group, 522(7554), pp. 98–101. doi: 10.1038/nature14488.

Apprill, A. *et al.* (2015) ‘Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton’, *Aquatic Microbial Ecology*, 75(2), pp. 129–137. doi: 10.3354/ame01753.

Arar, E. J. and Collins, G. B. (1997) ‘Method 445.0 In Vitro Determination of Chlorophyll a and Pheophytin ain Marine and Freshwater Algae by Fluorescence’. Available at: https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=309417 (Accessed: 18 May 2018).

Armbrust, E. V. (2009) ‘The life of diatoms in the world’s oceans’, *Nature*, 459(7244), pp. 185–192. doi: 10.1038/nature08057.

Atlas, R. M. (1975) ‘Effects of Temperature and Crude Oil Composition on Petroleum Biodegradation’, *Applied and Environmental Microbiology*, 30(3).

Azam, F. *et al.* (1983) ‘The Ecological Role of Water-Column Microbes in the Sea’, *Marine Ecology Progress Series*, 10, pp. 257–263. doi: 10.3354/meps010257.

Bach, L. T. *et al.* (2015) ‘A unifying concept of coccolithophore sensitivity to changing carbonate chemistry embedded in an ecological framework’, *Progress in Oceanography*. Pergamon, 135, pp. 125–138. doi: 10.1016/J.POCEAN.2015.04.012.

Baelum, J. *et al.* (2012) ‘Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill’, *Environmental Microbiology*. Wiley/Blackwell (10.1111), 14(9), pp. 2405–2416. doi: 10.1111/j.1462-2920.2012.02780.x.

Baker, M. (2012) ‘De novo genome assembly: what every biologist should know’, *Nature Methods*, 9(4), pp. 333–337. doi: 10.1038/nmeth.1935.

Balkwill, D. L., Fredrickson, J. K. and Romine, M. F. (2006) ‘Sphingomonas and Related Genera’, in *The Prokaryotes*. New York, NY: Springer New York, pp. 605–629. doi: 10.1007/0-387-30747-8_23.

Barcelos e Ramos, J. *et al.* (2007) ‘Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer Trichodesmium’, *Global Biogeochemical Cycles*. John Wiley & Sons, Ltd, 21(2), p. n/a-n/a. doi: 10.1029/2006GB002898.

Barcelos e Ramos, J., Müller, M. N. and Riebesell, U. (2010) ‘Short-term response of the coccolithophore Emiliania huxleyi to an abrupt change in seawater carbon dioxide concentrations’, *Biogeosciences*, 7(1), pp. 177–186. doi: 10.5194/bg-7-177-2010.

Bargiela, R. *et al.* (2015) ‘Bacterial population and biodegradation potential in chronically crude oil-contaminated marine sediments are strongly linked to temperature’, *Scientific Reports*. Nature Publishing Group, 5(November 2014), p.

11651. doi: 10.1038/srep11651.

Barry, J. P. *et al.* (2010) 'Atmospheric CO2 targets for ocean acidification perturbation experiments', in Riebesell, U. et al. (eds) *Guide to best practices for ocean acidification and data reporting*, pp. 53–66. Available at: https://boris.unibe.ch/4621/1/OA-Guide_Ch3_20100827.pdf (Accessed: 6 November 2018).

Beaufort, L. *et al.* (2011) 'Sensitivity of coccolithophores to carbonate chemistry and ocean acidification', *Nature*. Nature Publishing Group, 476(7358), pp. 80–83. doi: 10.1038/nature10295.

Behrenfeld, M. J. (2010) 'Abandoning Sverdrup's Critical Depth Hypothesis on phytoplankton blooms.', *Ecology*, 91(4), pp. 977–89. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20462113 (Accessed: 19 July 2018).

van Beilen, J. B. and Funhoff, E. G. (2007) 'Alkane hydroxylases involved in microbial alkane degradation', *Applied Microbiology and Biotechnology*. Springer-Verlag, 74(1), pp. 13–21. doi: 10.1007/s00253-006-0748-0.

Bell, W. H., Lang, J. M. and Mitchell, R. (1974) 'Selective stimulation of marine bacteria by algal extracellular products1', *Limnology and Oceanography*. Wiley-Blackwell, 19(5), pp. 833–839. doi: 10.4319/lo.1974.19.5.0833.

Berx, B. (2012) *The hydrography and circulation of the Faroe-Shetland Channel*, *Ocean Challenge*. Available at: https://blogs.gov.scot/marine-scotland/wp-content/uploads/sites/23/2014/09/berxb2012_final9_Faroe.pdf (Accessed: 22 May 2018).

Bett, B. J. (2001) 'UK Atlantic Margin Environmental Survey: Introduction and overview of bathyal benthic ecology', *Continental Shelf Research*. Pergamon, 21(8–10), pp. 917–956. doi: 10.1016/S0278-4343(00)00119-9.

Biegala, I. C. *et al.* (2002) 'Identification of Bacteria Associated with Dinoflagellates (Dinophyceae) Alexandrium ssp. Using Tyramide Signal Amplification-Fluorescent in situ Hybridization and Confocal Miscroscopy1', *Journal of Phycology*. Wiley/Blackwell

(10.1111), 38(2), pp. 404–411. doi: 10.1046/j.1529-8817.2002.01045.x.

Binark, N. *et al.* (2000) 'Oil Pollution of Marine Algae', *Bull. Environ. Contam. Toxicol*, 64, pp. 866–872. Available at: file:///Users/afiqdurr/Downloads/A3a-2.pdf (Accessed: 9 June 2018).

De Bodt, C. *et al.* (2010) 'Individual and interacting effects of pCO2 and temperature on Emiliania huxleyi calcification: Study of the calcite production, the coccolith morphology and the coccosphere size', *Biogeosciences*, 7(5), pp. 1401–1412. doi: 10.5194/bg-7-1401-2010.

Bolch, C. J. S., Subramanian, T. A. and Green, D. H. (2011) 'The Toxic Dinoflagellate Gymnodinium Catenatum (Dinophyceae) Requires Marine Bacteria for Growth1', *Journal of Phycology*. Wiley/Blackwell (10.1111), 47(5), pp. 1009–1022. doi: 10.1111/j.1529-8817.2011.01043.x.

Bordenstein, S. R. and Theis, K. R. (2015) 'Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes', *PLOS Biology*. Edited by M. K. Waldor. Public Library of Science, 13(8), p. e1002226. doi: 10.1371/journal.pbio.1002226.

Bozal, N. *et al.* (2003) 'Characterization of several Psychrobacter strains isolated from Antarctic environments and description of Psychrobacter luti sp. nov. and Psychrobacter fozii sp. nov.', *International Journal of Systematic and Evolutionary Microbiology*, 53, pp. 1093–1100. doi: 10.1099/ijs.0.02457-0.

BP Plc (2017) *BP announces start of production from Quad 204 project, west of Shetland | Press releases and latest news | Media | United Kingdom*. Available at: https://www.bp.com/en_gb/united-kingdom/media/press-releases/bp-announces-start-of-production-from-quad-204-project-west-of-shetland.html (Accessed: 27 May 2018).

Brakstad, O. G. and Lødeng, A. G. G. (2005) 'Microbial Diversity during Biodegradation of Crude Oil in Seawater from the North Sea', *Microbial Ecology*. Springer-Verlag, 49(1), pp. 94–103. doi: 10.1007/s00248-003-0225-6.

Bramucci, A. R. *et al.* (2018) 'The Bacterial Symbiont Phaeobacter inhibens Shapes the Life History of Its Algal Host Emiliania huxleyi', *Frontiers in Marine Science*. Frontiers, 5, p. 188. doi: 10.3389/fmars.2018.00188.

Bretter, I. *et al.* (2016) 'Climate Change Effects on Planktonic Bacterial Communities in the Ocean - From Structure and Function to Long-term and Large-scale Observations', in Marxsen, J. (ed.) *Climate Change and Microbial Ecology*. Caister Academic Press, pp. 23–39.

Brinkhoff, T. *et al.* (2004) 'Antibiotic production by a Roseobacter clade-affiliated species from the German Wadden Sea and its antagonistic effects on indigenous isolates.', *Applied and environmental microbiology*. American Society for Microbiology (ASM), 70(4), pp. 2560–5. doi: 10.1128/AEM.70.4.2560-2565.2003.

Burkhardt, S. *et al.* (2001) 'CO2 and HCO3- uptake in marine diatoms acclimated to different CO2 concentrations', *Limnology and Oceanography*. John Wiley & Sons, Ltd, 46(6), pp. 1378–1391. doi: 10.4319/lo.2001.46.6.1378.

Cai, W.-J. *et al.* (2011) 'Acidification of subsurface coastal waters enhanced by eutrophication', *Nature Geoscience*. Nature Publishing Group, 4(11), pp. 766–770. doi: 10.1038/ngeo1297.

Von Canstein, H. *et al.* (2002) 'Species diversity improves the efficiency of mercury-reducing biofilms under changing environmental conditions.', *Applied and environmental microbiology*. American Society for Microbiology (ASM), 68(6), pp. 2829–37. doi: 10.1128/AEM.68.6.2829-2837.2002.

Caporaso, J. G. *et al.* (2012) 'Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms', *The ISME Journal*. Nature Publishing Group, 6(8), pp. 1621–1624. doi: 10.1038/ismej.2012.8.

Caporaso, J. G. *et al.* (2018) *EMP 16S Illumina Amplicon Protocol*. Available at: https://www.protocols.io/view/emp-16s-illumina-amplicon-protocol-nuudeww (Accessed: 28 May 2018).

Chen, H. *et al.* (no date) '4 Years after the Deepwater Horizon Spill: Molecular Transformation of Macondo Well Oil in Louisiana Salt Marsh Sediments Revealed by FT-ICR Mass Spectrometry'. doi: 10.1021/acs.est.6b01156.

Choi, E. J. *et al.* (2010) 'Colwellia asteriadis sp. nov., a marine bacterium isolated from the starfish Asterias amurensis', *International Journal of Systematic and Evolutionary Microbiology*. Microbiology Society, 60(8), pp. 1952–1957. doi: 10.1099/ijs.0.016055-0.

Ciric, L., Philp, J. C. and Whiteley, A. S. (2010) 'Hydrocarbon utilization within a diesel-degrading bacterial consortium', *FEMS Microbiology Letters*. Wiley/Blackwell (10.1111), 303(2), pp. 116–122. doi: 10.1111/j.1574-6968.2009.01871.x.

Coelho, F. J. R. C. *et al.* (2013) 'Development and validation of an experimental life support system for assessing the effects of global climate change and environmental contamination on estuarine and coastal marine benthic communities', *Global Change Biology*, 19(8), pp. 2584–2595. doi: 10.1111/gcb.12227.

Coelho, F. J. R. C. *et al.* (2013) 'Interactive effects of global climate change and pollution on marine microbes: The way ahead', *Ecology and Evolution*. doi: 10.1002/ece3.565.

Coelho, F. J. R. C. *et al.* (2014) 'Unrevealing the interactive effects of climate change and oil contamination on lab-simulated estuarine benthic communities.', *Global change biology*, pp. 1–16. doi: 10.1111/gcb.12801.

Coelho, F. J. R. C. *et al.* (2015) 'Unraveling the interactive effects of climate change and oil contamination on laboratory-simulated estuarine benthic communities', *Global Change Biology*. doi: 10.1111/gcb.12801.

Coelho, F. J. R. C. *et al.* (2016) 'Multitaxon activity profiling reveals differential microbial response to reduced seawater pH and oil pollution', *Molecular Ecology*, pp. 4645–4659. doi: 10.1111/mec.13779.

Colebrook, J. M. (1982) 'Continuous plankton records: seasonal variations in the

distribution and abundance of plankton in the North Atlantic Ocean and the North Sea', *Journal of Plankton Research*. Oxford University Press, 4(3), pp. 435–462. doi: 10.1093/plankt/4.3.435.

Cottingham, K. L. *et al.* (2015) 'Cyanobacteria as biological drivers of lake nitrogen and phosphorus cycling', *Ecosphere Synthesis and Integration*, 6(1). doi: 10.1890/ES14-00174.1.

Coulon, F. *et al.* (2007) 'Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters', *Environmental Microbiology*, 9(1), pp. 177–186. doi: 10.1111/j.1462-2920.2006.01126.x.

Croft, M. T. *et al.* (2005) 'Algae acquire vitamin B12 through a symbiotic relationship with bacteria', *Nature*, 438(7064), pp. 90–93. doi: 10.1038/nature04056.

Crump, B. C., Armbrust, E. V and Baross, J. A. (1999) 'Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia river, its estuary, and the adjacent coastal ocean.', *Applied and environmental microbiology*, 65(7), pp. 3192–204. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10388721 (Accessed: 7 May 2018).

Dachs, J. *et al.* (2002) 'Oceanic biogeochemical controls on global dynamics of persistent organic pollutants.', *Environmental science & technology*, 36(20), pp. 4229–37. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12387392 (Accessed: 10 November 2018).

Dale, T., Rey, F. and Riddervold Heimdal, B. (1999) 'Seasonal development of phytoplankton at a high latitude oceanic site'. doi: 10.1080/00364827.1999.10807347.
Danbara, a. S. Y. and Shiraiwa, Y. (1999) 'The Requirement of Selenium for the Growth of Marine Coccolithophorids, Emiliania huxleyi, Gephyrocapsa oceanica and Helladosphaera sp. (Prymnesiophyceae)', *Plant Cell Physiol. 40(7): (1999)*, 40(40 (7)), pp. 762–766. doi: 10.1093/oxfordjournals.pcp.a029603.

Dandonneau, Y. *et al.* (2004) 'Seasonal and interannual variability of ocean color and composition of phytoplankton communities in the North Atlantic, equatorial Pacific and

South Pacific', *Deep Sea Research Part II: Topical Studies in Oceanography*. Pergamon, 51(1–3), pp. 303–318. doi: 10.1016/J.DSR2.2003.07.018.

Dao, G.-H. *et al.* (2018) 'Enhanced microalgae growth through stimulated secretion of indole acetic acid by symbiotic bacteria', *Algal Research*. Elsevier, 33, pp. 345–351. doi: 10.1016/J.ALGAL.2018.06.006.

Das, N. and Chandran, P. (2011) 'Microbial degradation of petroleum hydrocarbon contaminants: an overview.', *Biotechnology research international*. Hindawi Limited, 2011, p. 941810. doi: 10.4061/2011/941810.

Das, S. and Mangwani, N. (2015) 'Ocean acidification and marine microorganisms: responses and consequences', *Oceanologia*, 57(4), pp. 349–361. doi: 10.1016/j.oceano.2015.07.003.

Dason, J. S., Emma Huertas, I. and Colman, B. (2004) 'Source of Inorganic Carbon for Photosynthesis in Two Marine Dinoflagellate', *Journal of Phycology*. John Wiley & Sons, Ltd (10.1111), 40(2), pp. 285–292. doi: 10.1111/j.1529-8817.2004.03123.x.

Debes, H. H. (2000) *Plankton in the Faroe-Shetland Channel-a review*. Available at: http://projects.foib.fo/eia/Faroe_eia/Studies/Plankton_Final_1.pdf (Accessed: 4 November 2018).

Debes, H. H., Gallego, A. and Magen, C. (2007) 'Seasonal abundance and development of krill in the Faroe-Shetland Channel', *ICES* . Available at: file:///Users/afiqdurr/Downloads/Seasonal_abundance_and_development_of_krill_in_the.pdf (Accessed: 5 November 2018).

Dey, M. and Brummett, A. E. (2018) 'Isolation and Assays of Bacterial Dimethylsulfoniopropionate Lyases', *Methods in Enzymology*. Academic Press, 605, pp. 291–323. doi: 10.1016/BS.MIE.2018.02.020.

Diaz, M. R., Visscher, P. T. and Taylor, B. F. (1992) 'Metabolism of dimethylsulfoniopropionate and glycine betaine by a marine bacterium', *FEMS Microbiology Letters*. Oxford University Press, 96(1), pp. 61–65. doi: 10.1111/j.1574-

6968.1992.tb05394.x.

Dickson, A. G. (2011) ‘The carbon dioxide system in seawater: equilibrium chemistry and measurements’, in Riebesell, U. et al. (eds) *Guide to best practices for ocean acidification research and data reporting*. Directorate-General for Research anf Innovatin Environment. doi: 10.2777/66906.

Dickson, A. G. (2014) *Certificate of Analysis Reference material for oceanic CO 2 measurements, Batch*. Available at: http://cdiac.ornl.gov/oceans/Dickson_CRM/batches.html (Accessed: 28 May 2018).

Dickson, A. G., Sabine, C. L. and Christian, J. R. (2007) *Guide to Best Practices for Ocean CO2 Measurements, PICES Special Publication*. Available at: http://cdiac.ess-dive.lbl.gov/ftp/oceans/Handbook_2007/Guide_all_in_one.pdf (Accessed: 28 May 2018).

Dodds, W. K. *et al.* (2010) ‘Complex Community Interactions’, *Freshwater Ecology*. Academic Press, pp. 587–609. doi: 10.1016/B978-0-12-374724-2.00022-2.

Dong, C. *et al.* (2015) ‘Distribution of PAHs and the PAH-degrading bacteria in the deep-sea sediments of the high-latitude Arctic Ocean’, *Biogeosciences*, 12(7), pp. 2163–2177. doi: 10.5194/bg-12-2163-2015.

Dong, V. M. and Kim, D. (2018) *Cleaning Glassware | Protocol, JoVE Science Education Database*. Available at: https://www.jove.com/science-education/10342/cleaning-glassware (Accessed: 23 May 2018).

Dubinsky, E. A. *et al.* (2013) ‘Succession of Hydrocarbon-Degrading Bacteria in the Aftermath of the *Deepwater Horizon* Oil Spill in the Gulf of Mexico’, *Environmental Science & Technology*. American Chemical Society, 47(19), pp. 10860–10867. doi: 10.1021/es401676y.

Duran, R. and Cravo-Laureau, C. (2016) ‘Role of environmental factors and microorganisms in determining the fate of polycyclic aromatic hydrocarbons in the marine environment’, *FEMS Microbiology Reviews*. Edited by C. Brussaard. Oxford

University Press, 40(6), pp. 814–830. doi: 10.1093/femsre/fuw031.

Echeveste, P., Agustí, S. and Dachs, J. (2010) 'Cell size dependent toxicity thresholds of polycyclic aromatic hydrocarbons to natural and cultured phytoplankton populations', *Environmental Pollution*. Elsevier, 158(1), pp. 299–307. doi: 10.1016/J.ENVPOL.2009.07.006.

Egli, T. (1995) 'The Ecological and Physiological Significance of the Growth of Heterotrophic Microorganisms with Mixtures of Substrates', in. Springer, Boston, MA, pp. 305–386. doi: 10.1007/978-1-4684-7724-5_8.

Egli, T. (2010) 'How to live at very low substrate concentration', *Water Research*. Pergamon, 44(17), pp. 4826–4837. doi: 10.1016/J.WATRES.2010.07.023.

Falkowski, P. G. (1994) 'The role of phytoplankton photosynthesis in global biogeochemical cycles', *Photosynthesis Research*. Kluwer Academic Publishers, 39(3), pp. 235–258. doi: 10.1007/BF00014586.

Farag, S., Soliman, N. A. and Abdel-Fattah, Y. R. (2018) 'Statistical optimization of crude oil bio-degradation by a local marine bacterium isolate Pseudomonas sp. sp48', *Journal of Genetic Engineering and Biotechnology*. No longer published by Elsevier, 16(2), pp. 409–420. doi: 10.1016/J.JGEB.2018.01.001.

Feng, Y. *et al.* (2008) 'Interactive effects of increased pCO2, temperature and irradiance on the marine coccolithophore Emiliania huxleyi (Prymnesiophyceae)', *European Journal of Phycology*. Taylor & Francis Group, 43(1), pp. 87–98. doi: 10.1080/09670260701664674.

Feng, Y. *et al.* (2009) 'Effects of increased pCO2 and temperature on the North Atlantic spring bloom. I. The phytoplankton community and biogeochemical response', *Marine Ecology Progress Series Mar Ecol Prog Ser*, 388, pp. 13–25. doi: 10.3354/meps08133.

Ferguson, R. L., Buckley, E. N. and Palumbo, A. V (1984) 'Response of Marine Bacterioplankton to Differential Filtration and Confinement', *Appllied and Environmental Microbiology*, 47(1), pp. 49–55. Available at:

http://hahana.soest.hawaii.edu/agouroninstitutecourse/fergusonetal.pdf (Accessed: 28 May 2018).

Fiorini, S., Middelburg, J. and Gattuso, J. (2011) 'Effects of elevated CO2 partial pressure and temperature on the coccolithophore Syracosphaera pulchra', *Aquatic Microbial Ecology*, 64(3), pp. 221–232. doi: 10.3354/ame01520.

Fogg, G. E. and Thake, B. (1987) *Algal Cultures and Phytoplankton Ecology*. Third edit. Wisconsin: The University of Wisconsin Press.

Foster, Z. S. L., Sharpton, T. J. and Grünwald, N. J. (2017) 'Metacoder: An R package for visualization and manipulation of community taxonomic diversity data', *PLOS Computational Biology*. Edited by T. Poisot. Public Library of Science, 13(2), p. e1005404. doi: 10.1371/journal.pcbi.1005404.

Frada, M. *et al.* (2008) 'The "Cheshire Cat" escape strategy of the coccolithophore Emiliania huxleyi in response to viral infection.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 105(41), pp. 15944–9. doi: 10.1073/pnas.0807707105.

Friedland, K. D. *et al.* (2016) 'Seasonal phytoplankton blooms in the North Atlantic linked to the overwintering strategies of copepods', *Elementa: Science of the Anthropocene*. University of California Press, 4(0), p. 000099. doi: 10.12952/journal.elementa.000099.

Fritsche, W. and Hofrichter, M. (no date) 'Aerobic Degradation by Microorganisms'. Available at: http://marno.lecture.ub.ac.id/files/2012/05/BIODEGRADASI-AEROBIK-OLEH-MIKROORGANISME.pdf (Accessed: 30 April 2018).

Fu, F.-X. *et al.* (2007) 'Effects of Increased Temperature and CO2 on Photosynthesis, Growth, and Elemental Ratios in Marine Synechococcus and Prochlorococcus (Cyanobacteria)', *Journal of Phycology*. John Wiley & Sons, Ltd (10.1111), 43(3), pp. 485–496. doi: 10.1111/j.1529-8817.2007.00355.x.

Fu, F. *et al.* (2010) 'CO2 and phosphate availability control the toxicity of the harmful

bloom dinoflagellate Karlodinium veneficum', *Aquatic Microbial Ecology*, 59(1), pp. 55–65. doi: 10.3354/ame01396.

Fuentes, J. *et al.* (2016) 'Impact of Microalgae-Bacteria Interactions on the Production of Algal Biomass and Associated Compounds', *Marine Drugs*. Multidisciplinary Digital Publishing Institute, 14(5), p. 100. doi: 10.3390/md14050100.

Fukuda, S.-Y. *et al.* (2011) 'Compensatory response of the unicellular-calcifying alga Emiliania huxleyi (Coccolithophoridales, Haptophyta) to ocean acidification', *Journal of Oceanography*, 67, pp. 17–25. doi: 10.1007/s10872-011-0001-z.

Fukuda, S.-Y., Suzuki, Y. and Shiraiwa, Y. (2014) 'Difference in physiological responses of growth, photosynthesis and calcification of the coccolithophore Emiliania huxleyi to acidification by acid and CO2 enrichment.', *Photosynthesis research*. Springer, 121(2–3), pp. 299–309. doi: 10.1007/s11120-014-9976-9.

Gaard, E. (2000) 'Seasonal abundance and development of Calanus finmarchicus in relation to phytoplankton and hydrography on the Faroe Shelf', *ICES Journal of Marine Science*. Oxford University Press, 57(6), pp. 1605–1611. doi: 10.1006/jmsc.2000.0963.

Gallego, A. *et al.* (2018) 'Current status of deepwater oil spill modelling in the Faroe-Shetland Channel, Northeast Atlantic, and future challenges', *Marine Pollution Bulletin*. Pergamon, 127, pp. 484–504. doi: 10.1016/J.MARPOLBUL.2017.12.002.

Gallon, J. R. (2006) 'Reconciling the incompatible: N2 fixation And O2', *New Phytologist*. Wiley/Blackwell (10.1111), 122(4), pp. 571–609. doi: 10.1111/j.1469-8137.1992.tb00087.x.

Garcia, N. S. *et al.* (2011) 'Interactive Effects of Irradiance and CO2 on CO2 Fixation and N2 Fixation in the Diazotroph Trichodesmium Erythraeum (Cyanobacteria)', *Journal of Phycology*, 47(6), pp. 1292–1303. doi: 10.1111/j.1529-8817.2011.01078.x.

Garneau, M.-È. *et al.* (2016) 'Hydrocarbon biodegradation by Arctic sea-ice and sub-ice microbial communities during microcosm experiments, Northwest Passage (Nunavut, Canada)', *FEMS Microbiology Ecology*. Edited by M. Häggblom. Oxford University

Press, 92(10), p. fiw130. doi: 10.1093/femsec/fiw130.

Garza, D. R. and Dutilh, B. E. (2015) 'From cultured to uncultured genome sequences: metagenomics and modeling microbial ecosystems.', *Cellular and molecular life sciences : CMLS*. Springer, 72(22), pp. 4287–308. doi: 10.1007/s00018-015-2004-1.

Geng, H. and Belas, R. (2010) 'Expression of Tropodithietic Acid Biosynthesis Is Controlled by a Novel Autoinducer', *Journal of Bacteriology*, 192(17), pp. 4377–4387. doi: 10.1128/JB.00410-10.

Godoy, F. *et al.* (2003) 'Sphingopyxis chilensis sp. nov., a chlorophenol-degrading bacterium that accumulates polyhydroxyalkanoate, and transfer of Sphingomonas alaskensis to Sphingopyxis alaskensis comb. nov.', *International Journal of Systematic and Evolutionary Microbiology*. Microbiology Society, 53(2), pp. 473–477. doi: 10.1099/ijs.0.02375-0.

Gómez-Pereira, P. R. *et al.* (2012) 'Genomic content of uncultured Bacteroidetes from contrasting oceanic provinces in the North Atlantic Ocean', *Environmental Microbiology*. Wiley/Blackwell (10.1111), 14(1), pp. 52–66. doi: 10.1111/j.1462-2920.2011.02555.x.

Gong, J. *et al.* (2016) 'Protist-Bacteria Associations: Gammaproteobacteria and Alphaproteobacteria Are Prevalent as Digestion-Resistant Bacteria in Ciliated Protozoa.', *Frontiers in microbiology*. Frontiers Media SA, 7, p. 498. doi: 10.3389/fmicb.2016.00498.

Gontikaki, E. *et al.* (2018) 'Hydrocarbon-degrading bacteria in deep-water subarctic sediments (Faroe-Shetland Channel)', *Journal of Applied Microbiology*. doi: 10.1111/jam.14030.

González, J. *et al.* (2009) 'Effect of a simulated oil spill on natural assemblages of marine phytoplankton enclosed in microcosms', *Estuarine, Coastal and Shelf Science*. Academic Press, 83(3), pp. 265–276. doi: 10.1016/J.ECSS.2009.04.001.

Green, D. H. *et al.* (2006) 'Marinobacter algicola sp. nov., isolated from laboratory

cultures of paralytic shellfish toxin-producing dinoflagellates', *International Journal of Systematic and Evolutionary Microbiololgy*, 56(3), pp. 523–527. doi: 10.1099/ijs.0.63447-0.

Green, D. H. *et al.* (2015) 'Bacterial Diversity Associated with the Coccolithophorid Algae *Emiliania huxleyi* and *Coccolithus pelagicus* f. *braarudii*', *BioMed Research International*. Hindawi, 2015, pp. 1–15. doi: 10.1155/2015/194540.

Green, P. N. (2006) 'Methylobacterium', in *The Prokaryotes*. New York, NY: Springer New York, pp. 257–265. doi: 10.1007/0-387-30745-1_14.

Grimaud, R. *et al.* (2012) 'Genome sequence of the marine bacterium Marinobacter hydrocarbonoclasticus SP17, which forms biofilms on hydrophobic organic compounds.', *Journal of bacteriology*. American Society for Microbiology (ASM), 194(13), pp. 3539–40. doi: 10.1128/JB.00500-12.

Grossart, H.-P. *et al.* (2005) 'Marine diatom species harbour distinct bacterial communities', *Environmental Microbiology*. Wiley/Blackwell (10.1111), 7(6), pp. 860–873. doi: 10.1111/j.1462-2920.2005.00759.x.

Guillard, R. R. L. and Ryther, J. H. (1962) 'Studies of Marine Planktonic Diatoms: I. Cyclotella Nana Hustedt, and Detonula Confervacea (Cleve) Gran.', *Canadian Journal of Microbiology*. NRC Research Press Ottawa, Canada , 8(2), pp. 229–239. doi: 10.1139/m62-029.

Gustafsson, O., Gschwend, P. M. and Buesseler, K. 0 (1997) 'Using 234Th disequilibria to estimate the vertical removal rates of polycyclic aromatic hydrocarbons from the surface ocean', *Marine Chemistry*, 57(1). Available at: https://ac.els-cdn.com/S030442039700011X/1-s2.0-S030442039700011X-main.pdf?_tid=3da485e8-12bc-446e-89d5-a8eeefa12578&acdnat=1528541656_1b060279f8fe6f71baa861d4d3efb806 (Accessed: 9 June 2018).

Gutierrez, T. *et al.* (2012) 'Algiphilus aromaticivorans gen. nov., sp. nov., an aromatic hydrocarbon-degrading bacterium isolated from a culture of the marine dinoflagellate

Lingulodinium polyedrum, and proposal of Algiphilaceae fam. nov.', *International Journal of Systematic and Evolutionary Microbiology*. Microbiology Society, 62(Pt 11), pp. 2743–2749. doi: 10.1099/ijs.0.033324-0.

Gutierrez, T., Singleton, D. R., *et al.* (2013) 'Hydrocarbon-degrading bacteria enriched by the Deepwater Horizon oil spill identified by cultivation and DNA-SIP.', *The ISME journal*. Nature Publishing Group, 7(11), pp. 2091–104. doi: 10.1038/ismej.2013.98.

Gutierrez, T., Green, D. H., *et al.* (2013) 'Polycyclovorans algicola gen. nov., sp. nov., an aromatic-hydrocarbon-degrading marine bacterium found associated with laboratory cultures of marine phytoplankton.', *Applied and environmental microbiology*. American Society for Microbiology, 79(1), pp. 205–14. doi: 10.1128/AEM.02833-12.

Gutierrez, T. *et al.* (2014) 'Polycyclic aromatic hydrocarbon degradation of phytoplankton-associated Arenibacter spp. and description of Arenibacter algicola sp. nov., an aromatic hydrocarbon-degrading bacterium.', *Applied and environmental microbiology*. American Society for Microbiology, 80(2), pp. 618–28. doi: 10.1128/AEM.03104-13.

Hara, A., Syutsubo, K. and Harayama, S. (2003) 'Alcanivorax which prevails in oil-contaminated seawater exhibits broad substrate specificity for alkane degradation.', *Environmental microbiology*, 5(9), pp. 746–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12919410 (Accessed: 14 October 2018).

Harayama, S., Kasai, Y. and Hara, A. (2004) 'Microbial communities in oil-contaminated seawater', *Current Opinion in Biotechnology*, 15(3), pp. 205–214. doi: 10.1016/j.copbio.2004.04.002.

Hassenrück, C. *et al.* (2016) 'Quantification of the effects of ocean acidification on sediment microbial communities in the environment: the importance of ecosystem approaches.', *FEMS microbiology ecology*. Oxford University Press, 92(5), p. fiw027. doi: 10.1093/femsec/fiw027.

Head, I. M., Jones, D. M. and Röling, W. F. M. (2006) 'Marine microorganisms make a meal of oil.', *Nature reviews. Microbiology*, 4(3), pp. 173–82. doi:

10.1038/nrmicro1348.

Head, R. N. *et al.* (1998) 'The hydrography and biology of a bloom of the coccolithophorid Emiliania huxleyi in the northern North Sea', *Journal of Sea Research*. Elsevier, 39(3–4), pp. 255–266. doi: 10.1016/S1385-1101(97)00060-9.

Hemalatha, S. and Veeramanikandan, P. (2011) 'Characterization of Aromatic Hydrocarbon Degrading Bacteria from Petroleum Contaminated Sites', *Journal of Environmental Protection*, 2, pp. 243–254. doi: 10.4236/jep.2011.23028.

Hibbing, M. E. *et al.* (2010) 'Bacterial competition: surviving and thriving in the microbial jungle.', *Nature reviews. Microbiology*. NIH Public Access, 8(1), pp. 15–25. doi: 10.1038/nrmicro2259.

Hjorth, M., Forbes, V. and Dahllöf, I. (2008) 'Plankton stress responses from PAH exposure and nutrient enrichment', *Marine Ecology Progress Series*, 363, pp. 121–130. doi: 10.3354/meps07470.

Holligan, P. M., Charalampopoulou, A. and Hutson, R. (2010) 'Seasonal distributions of the coccolithophore, Emiliania huxleyi, and of particulate inorganic carbon in surface waters of the Scotia Sea', *Journal of Marine Systems*. Elsevier, 82(4), pp. 195–205. doi: 10.1016/J.JMARSYS.2010.05.007.

Hong, S. *et al.* (2009) 'Polymerase chain reaction primers miss half of rRNA microbial diversity', *The ISME Journal*. Nature Publishing Group, 3(12), pp. 1365–1373. doi: 10.1038/ismej.2009.89.

Hong, Z. *et al.* (2015) 'Sulfitobacter pseudonitzschiae sp. nov., isolated from the toxic marine diatom Pseudo-nitzschia multiseries', *International Journal of Systematic and Evolutionary Microbiology.*, 65(Pt 1), pp. 95–100. doi: 10.1099/ijs.0.064972-0.

Hönisch, B. *et al.* (2012) 'The Geological Record of Ocean Acidification'. doi: 10.1126/science.1208277.

Hopkinson, B. M. *et al.* (2011) 'Efficiency of the CO2-concentrating mechanism of

diatoms', *Proceedings of the National Academy of Sciences*. National Academy of Sciences, 108(10), pp. 3830–3837. doi: 10.1073/PNAS.1018062108.

Hoppe, C. J. M. *et al.* (2013) 'Iron Limitation Modulates Ocean Acidification Effects on Southern Ocean Phytoplankton Communities', *PLoS ONE*. Edited by E. V. Thuesen. Public Library of Science, 8(11), p. e79890. doi: 10.1371/journal.pone.0079890.

Huang, Y.-J. *et al.* (2011) 'The chronic effects of oil pollution on marine phytoplankton in a subtropical bay, China', *Environmental Monitoring and Assessment*, 176(1–4), pp. 517–530. doi: 10.1007/s10661-010-1601-6.

Huang, Y. *et al.* (2018) 'Effects of increasing atmospheric CO 2 on the marine phytoplankton and bacterial metabolism during a bloom: A coastal mesocosm study', *Science of the Total Environment*, 633, pp. 618–629. doi: 10.1016/j.scitotenv.2018.03.222.

Hutchins, D. A. *et al.* (2007) 'CO2 control of Trichodesmium N2 fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present, and future ocean biogeochemistry', *Limnology and Oceanography*. John Wiley & Sons, Ltd, 52(4), pp. 1293–1304. doi: 10.4319/lo.2007.52.4.1293.

Hutchins, D. A. *et al.* (2013) 'Taxon-specific response of marine nitrogen fixers to elevated carbon dioxide concentrations', *Nature Geoscience*. Nature Publishing Group, 6(9), pp. 790–795. doi: 10.1038/ngeo1858.

Iglesias-Rodriguez, D. M. *et al.* (2008) 'Phytoplankton Calcification in a High-CO 2 World', *Science*, 320, pp. 336–340. Available at: http://science.sciencemag.org.ezproxy1.hw.ac.uk/content/sci/320/5874/336.full.pdf (Accessed: 27 June 2018).

Ivanova, E. P. *et al.* (2004) 'Sulfitobacter delicatus sp. nov. and Sulfitobacter dubius sp. nov., respectively from a starfish (Stellaster equestris) and sea grass (Zostera marina)', *International Journal of Systematic and Evolutionary Microbiology*, 54, pp. 475–480. doi: 10.1099/ijs.0.02654-0.

Jakob, I., Weggenmann, F. and Posten, C. (2018) ‘Cultivation of Emiliania huxleyi for coccolith production’, *Algal Research*, 31, pp. 47–59. Available at: file:///Users/afiqdurr/Downloads/CultivationofEmilianiahuxleyiforcoccolithproduction.pdf (Accessed: 20 June 2019).

Jessup, C. M. *et al.* (2004) ‘Big questions, small worlds: microbial model systems in ecology’. doi: 10.1016/j.tree.2004.01.008. JNCC (2018) *Supplementary Advice on Conservation Objectives for North-East Faroe-Shetland Channel Nature Conservation Marine Protected Area*. Available at: http://jncc.defra.gov.uk/pdf/NEFSC_SACO_v1.0.pdf (Accessed: 5 November 2018).

Joint, I., Doney, S. C. and Karl, D. M. (2010) ‘Will ocean acidification affect marine microbes?’, *The ISME Journal*, 5(1), pp. 1–7. doi: 10.1038/ismej.2010.79.

Joye, S. B. and Kleindienst, S. (2016) ‘Responses of Microbial Communities to Hydrocarbon Exposures’, (September). doi: 10.5670/oceanog.2016.78.

Jurgens, K. and Gude, H. (1994) *The potential importance of grazing-resistant bacteria in planktonic systems*, *Marine Ecology Progress Series Mar. Ecol. Prog. Ser*. Available at: https://www.int-res.com/articles/meps/112/m112p169.pdf (Accessed: 24 June 2019).

Kahfi, K. (2018) *Balikpapan oil spill: What we know and don’t know - National - The Jakarta Post*, *The Jakarta Post*. Available at: http://www.thejakartapost.com/news/2018/04/11/qa-balikpapan-oil-spill-what-we-know-and-dont-know.html (Accessed: 10 November 2018).

Kang, H.-J., Lee, S.-Y. and Kwon, J.-H. (2016) ‘Physico-chemical properties and toxicity of alkylated polycyclic aromatic hydrocarbons’, *Journal of Hazardous Materials*, 312, pp. 200–207. doi: 10.1016/j.jhazmat.2016.03.051.

Kappell, A. D. *et al.* (2014) ‘The polycyclic aromatic hydrocarbon degradation potential of Gulf of Mexico native coastal microbial communities after the Deepwater Horizon oil spill’, *Frontiers in Microbiology*, 5(May), pp. 1–13. doi: 10.3389/fmicb.2014.00205.

Kawahara, K. *et al.* (2001) ‘Structural analysis of a new glycosphingolipid from the

lipopolysaccharide-lacking bacterium Sphingomonas adhaesiva', *Carbohydrate Research*. Elsevier, 333(1), pp. 87–93. doi: 10.1016/S0008-6215(01)00111-2.

Kertesz, M. A. and Kawasaki, A. (2010) 'Hydrocarbon-Degrading Sphingomonads: Sphingomonas, Sphingobium, Novosphingobium, and Sphingopyxis', in *Handbook of Hydrocarbon and Lipid Microbiology*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 1693–1705. doi: 10.1007/978-3-540-77587-4_119.

Kim, J. *et al.* (2014) 'Draft Genome Sequence of Sphingopyxis sp. Strain MWB1, a Crude-Oil-Degrading Marine Bacterium.', *Genome announcements*. American Society for Microbiology (ASM), 2(6). doi: 10.1128/genomeA.01256-14.

Kim, Y.-O. *et al.* (2013) 'Colwellia meonggei sp. nov., a novel gammaproteobacterium isolated from sea squirt Halocynthia roretzi', *Antonie van Leeuwenhoek*. Springer Netherlands, 104(6), pp. 1021–1027. doi: 10.1007/s10482-013-0022-2.

Kimes, N. E. *et al.* (2014) 'Microbial transformation of the Deepwater Horizon oil spillâ€"past, present, and future perspectives', *Frontiers in Microbiology*, 5(November), pp. 1–11. doi: 10.3389/fmicb.2014.00603.

Kleindienst, S. *et al.* (2016) 'Diverse, rare microbial taxa responded to the Deepwater Horizon deep-sea hydrocarbon plume', *The ISME Journal*, 10(2), pp. 400–415. doi: 10.1038/ismej.2015.121.

Koshikawa, H. *et al.* (2007) 'Effect of the water-soluble fraction of diesel oil on bacterial and primary production and the trophic transfer to mesozooplankton through a microbial food web in Yangtze estuary, China', *Estuarine, Coastal and Shelf Science*. Academic Press, 71(1–2), pp. 68–80. doi: 10.1016/J.ECSS.2006.08.008.

Kostka, J. E. *et al.* (2011) 'Hydrocarbon-degrading bacteria and the bacterial community response in gulf of Mexico beach sands impacted by the deepwater horizon oil spill.', *Applied and environmental microbiology*. American Society for Microbiology, 77(22), pp. 7962–74. doi: 10.1128/AEM.05402-11.

Kozich, J. J. *et al.* (2013) 'Development of a dual-index sequencing strategy and

curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform.', *Applied and environmental microbiology*. American Society for Microbiology (ASM), 79(17), pp. 5112–20. doi: 10.1128/AEM.01043-13.

Kranz, S. A. *et al.* (2009) 'Carbon acquisition by Trichodesmium: the effect of pCO2 and diurnal changes', *Limnology and Oceanography*. John Wiley & Sons, Ltd, 54(2), pp. 548–559. doi: 10.4319/lo.2009.54.2.0548.

Kranz, S. A. *et al.* (2010) 'Combined effects of CO2 and light on the N2-fixing cyanobacterium Trichodesmium IMS101: physiological responses.', *Plant physiology*. American Society of Plant Biologists, 154(1), pp. 334–45. doi: 10.1104/pp.110.159145.

Kroeker, K. J. *et al.* (2011) 'Response to technical comment on "meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms"', *Ecology Letters*, 14(9), pp. 1–3. doi: 10.1111/j.1461-0248.2011.01665.x.

Krumhardt, K. M. *et al.* (2016) 'Apparent increase in coccolithophore abundance in the subtropical North Atlantic from 1990 to 2014', *Biogeosciences*, 13, pp. 1163–1177. doi: 10.5194/bg-13-1163-2016.

Kusk, K. O. (1978) 'Effects of Crude Oil and Aromatic Hydrocarbons on the Photosynthesis of the Diatom Nitzschia palea', *Physiologia Plantarum*. Wiley/Blackwell (10.1111), 43(1), pp. 1–6. doi: 10.1111/j.1399-3054.1978.tb01558.x.

Lagier, J.-C. *et al.* (2016) 'Culture of previously uncultured members of the human gut microbiota by culturomics', *Nature Microbiology*. Nature Publishing Group, 1(12), p. 16203. doi: 10.1038/nmicrobiol.2016.203.

LaMontagne, M. G. *et al.* (2004) 'Bacterial diversity in marine hydrocarbon seep sediments', *Environmental Microbiology*. Wiley/Blackwell (10.1111), 6(8), pp. 799–808. doi: 10.1111/j.1462-2920.2004.00613.x.

Langer, G. *et al.* (2006) 'Species-specific responses of calcifying algae to changing seawater carbonate chemistry'. doi: 10.1029/2005GC001227.

Langer, G. *et al.* (2009) ‘Strain-specific responses of Emiliania huxleyi to changing seawater carbonate chemistry’, pp. 2637–2646.

Langer, G. and Bode, M. (2011) ‘CO2 mediation of adverse effects of seawater acidification in Calcidiscus leptoporus’, *Geochemistry, Geophysics, Geosystems*. John Wiley & Sons, Ltd, 12(5), p. n/a-n/a. doi: 10.1029/2010GC003393.

Lee, C. K. *et al.* (2012) ‘Groundtruthing Next-Gen Sequencing for Microbial Ecology–Biases and Errors in Community Structure Estimates from PCR Amplicon Pyrosequencing’, *PLoS ONE*. Edited by J. A. Gilbert. Public Library of Science, 7(9), p. e44224. doi: 10.1371/journal.pone.0044224.

Lefebvre, S. C. *et al.* (2012) ‘Nitrogen source and pCO2 synergistically affect carbon allocation, growth and morphology of the coccolithophore Emiliania huxleyi: potential implications of ocean acidification for the carbon cycle’, *Global Change Biology*. John Wiley & Sons, Ltd (10.1111), 18(2), pp. 493–503. doi: 10.1111/j.1365-2486.2011.02575.x.

Lemkau, K. L. *et al.* (2014) ‘Molecular Evidence of Heavy-Oil Weathering Following the M/V Cosco Busan Spill: Insights from Fourier Transform Ion Cyclotron Resonance Mass Spectrometry’, *Environmental Science & Technology*. doi: 10.1021/es403787u.

Levitan, O. *et al.* (2007) ‘Elevated CO2 enhances nitrogen fixation and growth in the marine cyanobacterium Trichodesmium’, *Global Change Biology*. John Wiley & Sons, Ltd (10.1111), 13(2), pp. 531–538. doi: 10.1111/j.1365-2486.2006.01314.x.

Levitan, O., Kranz, S. A., *et al.* (2010) ‘Combined effects of CO2 and light on the N2-fixing cyanobacterium Trichodesmium IMS101: a mechanistic view.’, *Plant physiology*. American Society of Plant Biologists, 154(1), pp. 346–56. doi: 10.1104/pp.110.159285.

Levitan, O., Sudhaus, S., *et al.* (2010) ‘The influence of pCO2 and temperature on gene expression of carbon and nitrogen pathways in Trichodesmium IMS101.’, *PloS one*. Public Library of Science, 5(12), p. e15104. doi: 10.1371/journal.pone.0015104.

Lévy, M. *et al.* (2005) ‘Production regimes in the northeast Atlantic: A study based on

Sea-viewing Wide Field-of-view Sensor (SeaWiFS) chlorophyll and ocean general circulation model mixed layer depth', *Journal of Geophysical Research*. Wiley-Blackwell, 110(C7), p. C07S10. doi: 10.1029/2004JC002771.

Li, Y., Xu, J. and Gao, K. (2014) 'Light-Modulated Responses of Growth and Photosynthetic Performance to Ocean Acidification in the Model Diatom Phaeodactylum tricornutum', *PLoS ONE*. Edited by W. L. Araujo. Public Library of Science, 9(5), p. e96173. doi: 10.1371/journal.pone.0096173.

Liu, X. *et al.* (2017) 'Effects of elevated CO2 on phytoplankton during a mesocosm experiment in the southern eutrophicated coastal water of China.', *Scientific reports*. Nature Publishing Group, 7(1), p. 6868. doi: 10.1038/s41598-017-07195-8.

Loeschcke, A. and Thies, S. (2015) 'Pseudomonas putida-a versatile host for the production of natural products.', *Applied microbiology and biotechnology*. Springer, 99(15), pp. 6197–214. doi: 10.1007/s00253-015-6745-4.

Logares, R. *et al.* (2014) 'Metagenomic 16S rDNA Illumina tags are a powerful alternative to amplicon sequencing to explore diversity and structure of microbial communities', *Environmental Microbiology*, 16(9), pp. 2659–2671. doi: 10.1111/1462-2920.12250.

Lomas, M. W. *et al.* (2012) 'Effect of ocean acidification on cyanobacteria in the subtropical North Atlantic', *Aquatic Microbial Ecology*, 66, pp. 211–222. doi: 10.3354/ame01576.

Longhurst, A. (1995) 'Seasonal cycles of pelagic production and consumption', *Progress in Oceanography*. Pergamon, 36(2), pp. 77–167. doi: 10.1016/0079-6611(95)00015-1.

Lu, Z. *et al.* (2012) 'Microbial gene functions enriched in the Deepwater Horizon deep-sea oil plume', *The ISME Journal*. Nature Publishing Group, 6(2), pp. 451–460. doi: 10.1038/ismej.2011.91.

Lupette, J. *et al.* (2016) 'Marinobacter Dominates the Bacterial Community of the

Ostreococcus tauri Phycosphere in Culture', *Frontiers in Microbiology*. Frontiers, 7, p. 1414. doi: 10.3389/fmicb.2016.01414.

Ma, Y. *et al.* (2013) 'Deposition of polycyclic aromatic hydrocarbons in the North Pacific and the Arctic', *Journal of Geophysical Research: Atmospheres*. Wiley-Blackwell, 118(11), pp. 5822–5829. doi: 10.1002/jgrd.50473.

Mackey, K. R. M. *et al.* (2013) 'Effect of Temperature on Photosynthesis and Growth in Marine Synechococcus spp.' doi: 10.1104/pp.113.221937.

Mackey, K. R. M. *et al.* (2015) 'Response of Photosynthesis to Ocean Acidification', *Oceanography* . The Oceanography Society, 28(2), pp. 74–91. doi: 10.5670/oceanog.2015.33.

Main, C. E. *et al.* (2017) 'Simulating pathways of subsurface oil in the Faroe–Shetland Channel using an ocean general circulation model', *Marine Pollution Bulletin*. Pergamon, 114(1), pp. 315–326. doi: 10.1016/J.MARPOLBUL.2016.09.041.

Malin, G. (1996) 'The Role of DMSP and DMS in the Global Sulfur Cycle and Climate Regulation', in *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds*. Boston, MA: Springer US, pp. 177–189. doi: 10.1007/978-1-4613-0377-0_16.

Mari, X. (2008) 'Does ocean acidification induce an upward flux of marine aggregates?', *Biogeosciences Discussions*, 5(2), pp. 1631–1654. doi: 10.5194/bgd-5-1631-2008.

Marín-Spiotta, E. *et al.* (2014) 'Paradigm shifts in soil organic matter research affect interpretations of aquatic carbon cycling: transcending disciplinary and ecosystem boundaries', *Biogeochemistry*. Springer International Publishing, 117(2–3), pp. 279–297. doi: 10.1007/s10533-013-9949-7.

Marine Scotland (2017a) *Taking the Sea-Bird to the Faroe-Shetland Channel*. Available at: https://blogs.gov.scot/marine-scotland/2017/12/08/taking-the-sea-bird-to-the-faroe-shetland-channel/ (Accessed: 23 May 2018).

Marine Scotland (2017b) *UK Fishing Intensity Associated with Oil and Gas Pipelines (2007-2015) | Marine Scotland Data Publications*. Available at: https://data.marine.gov.scot/dataset/uk-fishing-intensity-associated-oil-and-gas-pipelines-2007-2015-0 (Accessed: 29 May 2018).

Marinho, P. R. *et al.* (2009) 'Marine Pseudomonas putida: a potential source of antimicrobial substances against antibiotic-resistant bacteria', *Memórias do Instituto Oswaldo Cruz*. Fundação Oswaldo Cruz, 104(5), pp. 678–682. doi: 10.1590/S0074-02762009000500002.

Martinez, E. *et al.* (2011) 'Phytoplankton spring and fall blooms in the North Atlantic in the 1980s and 2000s', *Journal of Geophysical Research*. Wiley-Blackwell, 116(C11), p. C11029. doi: 10.1029/2010JC006836.

Mason, O. U. *et al.* (2014) 'Single-cell genomics reveals features of a Colwellia species that was dominant during the Deepwater Horizon oil spill.', *Frontiers in microbiology*. Frontiers Media SA, 5, p. 332. doi: 10.3389/fmicb.2014.00332.

Matsuda, Y., Hara, T. and Colman, B. (2001) 'Regulation of the induction of bicarbonate uptake by dissolved CO2 in the marine diatom, Phaeodactylum tricornutum', *Plant, Cell and Environment*. John Wiley & Sons, Ltd (10.1111), 24(6), pp. 611–620. doi: 10.1046/j.1365-3040.2001.00702.x.

Mayers, T. J. *et al.* (2016) 'A Bacterial Pathogen Displaying Temperature-Enhanced Virulence of the Microalga Emiliania huxleyi.', *Frontiers in microbiology*. Frontiers Media SA, 7, p. 892. doi: 10.3389/fmicb.2016.00892.

McGenity, T. J. *et al.* (2012) 'Marine crude-oil biodegradation: a central role for interspecies interactions.', *Aquatic biosystems*. BioMed Central, 8(1), p. 10. doi: 10.1186/2046-9063-8-10.

McKenna, A. M. *et al.* (2013) 'Expansion of the Analytical Window for Oil Spill Characterization by Ultrahigh Resolution Mass Spectrometry: Beyond Gas Chromatography', *Environmental Science & Technology*. American Chemical Society,

47(13), pp. 7530–7539. doi: 10.1021/es305284t.

Mcnutt, M. K. *et al.* (2011) 'Review of flow rate estimates of the Deepwater Horizon oil spill', *PNAS*, 109(50). doi: 10.1073/pnas.1112139108/-/DCSupplemental.

McQuatters-Gollop, A. *et al.* (2007) 'Spatial patterns of diatom and dinoflagellate seasonal cycles in the NE Atlantic Ocean', *Mar Ecol Prog Ser*, 339, pp. 301–306. Available at: www.int-res.com (Accessed: 3 November 2018).

Medigue, C. *et al.* (2005) 'Coping with cold: The genome of the versatile marine Antarctica bacterium Pseudoalteromonas haloplanktis TAC125', *Genome Research*, 15(10), pp. 1325–1335. doi: 10.1101/gr.4126905.

Methé, B. A. *et al.* (2005) *The psychrophilic lifestyle as revealed by the genome sequence of Colwellia psychrerythraea 34H through genomic and proteomic analyses*. Available at: www.pnas.orgcgidoi10.1073pnas.0504766102 (Accessed: 13 October 2018).

Mignot, A., Ferrari, R. and Claustre, H. (2018) 'Floats with bio-optical sensors reveal what processes trigger the North Atlantic bloom', *Nature Communications*. Nature Publishing Group, 9(1), p. 190. doi: 10.1038/s41467-017-02143-6.

Miller, M. B. and Bassler, B. L. (2001) 'Quorum Sensing in Bacteria', *Annual Review of Microbiology*, 55(1), pp. 165–199. doi: 10.1146/annurev.micro.55.1.165.

Mishamandani, S. *et al.* (2015) 'Response of the bacterial community associated with a cosmopolitan marine diatom to crude oil shows a preference for the biodegradation of aromatic hydrocarbons.', *Environmental microbiology*. doi: 10.1111/1462-2920.12988.

Mukherjee, A. *et al.* (2017) 'Bioinformatic Approaches Including Predictive Metagenomic Profiling Reveal Characteristics of Bacterial Response to Petroleum Hydrocarbon Contamination in Diverse Environments', *Scientific Reports*, 7. doi: 10.1038/s41598-017-01126-3.

Müller, M. N. *et al.* (2012) 'Influence of CO2 and nitrogen limitation on the coccolith

volume of Emiliania huxleyi (Haptophyta)', *Biogeosciences*, 9(10), pp. 4155–4167. doi: 10.5194/bg-9-4155-2012.

Müller, M. N., Schulz, K. G. and Riebesell, U. (2010) 'Effects of long-term high CO2 exposure on two species of coccolithophores', *Biogeosciences*, 7(3), pp. 1109–1116. doi: 10.5194/bg-7-1109-2010.

Muñoz, R. and Guieysse, B. (2006) 'Algal–bacterial processes for the treatment of hazardous contaminants: A review', *Water Research*. Pergamon, 40(15), pp. 2799–2815. doi: 10.1016/J.WATRES.2006.06.011.

Myers, J. (1953) 'Growth characteristics of algae in relation to problems of mass culture', in J. S. Burlew (ed.) *Algal Culture From Laboratory To Pilot Plant*. Washington, D. C.: Carnegie Institution of Washington Publication , pp. 37–54. Available at: https://s3.amazonaws.com/academia.edu.documents/36959176/algal_culture.pdf?AWSAccessKeyId=AKIAIWOWYYGZ2Y53UL3A&Expires=1527499097&Signature=9ebKyrr05RLH7jBpP9qLt8DrsYo%3D&response-content-disposition=inline%3Bfilename%3DEdited_by.pdf#page=48 (Accessed: 28 May 2018).

Nakicenovic, N. and Swart, R. (2000) *Special Report on Emissions Scenarios: A Special Report of Working Group III of tiie Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press. Available at: https://www.ipcc.ch/pdf/special-reports/emissions_scenarios.pdf (Accessed: 7 November 2018).

Nelson, D. M. *et al.* (1995) 'Production and dissolution of biogenic silica in the ocean: Revised global estimates, comparison with regional data and relationship to biogenic sedimentation', *Global Biogeochemical Cycles*. John Wiley & Sons, Ltd, 9(3), pp. 359–372. doi: 10.1029/95GB01070.

Nikolaou, A. *et al.* (2009) 'Levels and toxicity of polycyclic aromatic hydrocarbons in marine sediments', *TrAC Trends in Analytical Chemistry*. Elsevier, 28(6), pp. 653–664. doi: 10.1016/J.TRAC.2009.04.004.

Nimer, N. A., Brownlee, C. and Merrett, M. J. (1994) ‘Carbon dioxide availability, intracellular pH and growth rate of the coccolithophore Emiliania huxleyi’, *Marine Ecology Progress Series*. Inter-Research Science Center, pp. 257–262. doi: 10.2307/24846191.

Obata, T. *et al.* (2013) ‘Gas-Chromatography Mass-Spectrometry (GC-MS) Based Metabolite Profiling Reveals Mannitol as a Major Storage Carbohydrate in the Coccolithophorid Alga Emiliania huxleyi’, *Metabolites*. Multidisciplinary Digital Publishing Institute, 3(1), pp. 168–184. doi: 10.3390/metabo3010168.

OGA Offshore FieldDets WGS84 (2018) *Oil and Gas Authority Open Data*. Available at: http://data-ogauthority.opendata.arcgis.com/datasets?q=oga offshore (Accessed: 23 May 2018).

Olson, G. M. *et al.* (2017) ‘Effect of Corexit 9500A on Mississippi Canyon crude oil weathering patterns using artificial and natural seawater’, *Heliyon*. Elsevier, 3(3), p. e00269. doi: 10.1016/j.heliyon.2017.e00269.

Orr, J. C. *et al.* (2005) ‘Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms’, *Nature*. Nature Publishing Group, 437(7059), pp. 681–686. doi: 10.1038/nature04095.

Ozhan, K. and Bargu, S. (2014) ‘Distinct responses of Gulf of Mexico phytoplankton communities to crude oil and the dispersant corexit® Ec9500A under different nutrient regimes’, *Ecotoxicology*. Springer US, 23(3), pp. 370–384. doi: 10.1007/s10646-014-1195-9.

Ozhan, K., Parsons, M. L. and Bargu, S. (2014) ‘How Were Phytoplankton Affected by the Deepwater Horizon Oil Spill?’, *BioScience*, 64(9), pp. 829–836. doi: 10.1093/biosci/biu117.

Pachauri, R. K. *et al.* (2014) *Climate Change 2014 Synthesis Report*. Available at: http://www.ipcc.ch/pdf/assessment-report/ar5/syr/SYR_AR5_FINAL_full.pdf.

Paerl, H., W. (1982) ‘Interactions with bacteria’, in *The Biology of Cyanobacteria*.

Blackwell Scientific Publications Ltd, pp. 441–446.

Paerl, H. W. and Millie, D. F. (1996) 'Physiological ecology of toxic aquatic cyanobacteria', *Phycologia*. The International Phycological Society Phycologia Business Office, Allen Press, 810 East 10th Street, P.O. Box 1897, Lawrence, KS 66044-8897 , 35(6S), pp. 160–167. doi: 10.2216/i0031-8884-35-6S-160.1.

Paerl, H. W. and Otten, T. G. (2013) 'Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls', *Microbial Ecology*, 65(4), pp. 995–1010. doi: 10.1007/s00248-012-0159-y.

Paerl, H. W. and Paul, V. J. (2012) 'Climate change: Links to global expansion of harmful cyanobacteria', *Water Research*. Pergamon, 46(5), pp. 1349–1363. doi: 10.1016/J.WATRES.2011.08.002.

Parab, S. R. *et al.* (2008) 'Effect of Bombay high crude oil and its water-soluble fraction on growth and metabolism of diatom Thalassiosira sp.' NISCAIR, CSIR. Available at: http://drs.nio.org/drs/handle/2264/1496 (Accessed: 10 November 2018).

Passow, U. (2002) 'Transparent exopolymer particles (TEP) in aquatic environments', *Progress in Oceanography*, 55, pp. 287–333. Available at: www.elsevier.com/locate/pocean (Accessed: 14 May 2018).

Paul, C., Matthiessen, B. and Sommer, U. (2015) 'Warming, but not enhanced CO2 concentration, quantitatively and qualitatively affects phytoplankton biomass', *Marine Ecology Progress Series*, 528, pp. 39–51. doi: 10.3354/meps11264.

Paul, C. and Pohnert, G. (2011) 'Interactions of the Algicidal Bacterium Kordia algicida with Diatoms: Regulated Protease Excretion for Specific Algal Lysis', *PLoS ONE*. Edited by T. Evens. Public Library of Science, 6(6), p. e21032. doi: 10.1371/journal.pone.0021032.

Paul, C. and Pohnert, G. (2013) 'Induction of Protease Release of the Resistant Diatom Chaetoceros didymus in Response to Lytic Enzymes from an Algicidal Bacterium', *PLoS ONE*. Edited by V. N. Uversky. Public Library of Science, 8(3), p. e57577. doi:

10.1371/journal.pone.0057577.

Peeters, F. *et al.* (2007) 'Earlier onset of the spring phytoplankton bloom in lakes of the temperate zone in a warmer climate', *Global Change Biology*. Wiley/Blackwell (10.1111), 13(9), pp. 1898–1909. doi: 10.1111/j.1365-2486.2007.01412.x.

Perez Calderon, L. J. *et al.* (2018) 'Bacterial Community Response in Deep Faroe-Shetland Channel Sediments Following Hydrocarbon Entrainment With and Without Dispersant Addition', *Frontiers in Marine Science*. Frontiers, 5, p. 159. doi: 10.3389/fmars.2018.00159.

Pernthaler, J. and Amann, R. (2005) 'Fate of heterotrophic microbes in pelagic habitats: focus on populations.', *Microbiology and molecular biology reviews : MMBR*. American Society for Microbiology, 69(3), pp. 440–61. doi: 10.1128/MMBR.69.3.440-461.2005.

Pinto, A. J. and Raskin, L. (2012) 'PCR Biases Distort Bacterial and Archaeal Community Structure in Pyrosequencing Datasets', *PLoS ONE*. Edited by S. Bertilsson. Public Library of Science, 7(8), p. e43093. doi: 10.1371/journal.pone.0043093.

Plattner, G. K. *et al.* (2001) 'Feedback mechanisms and sensitivities of ocean carbon uptake under global warming', *Tellus B*. Wiley/Blackwell (10.1111), 53(5), pp. 564–592. doi: 10.1034/j.1600-0889.2001.530504.x.

Pomeroy, L. *et al.* (2007) 'The Microbial Loop', *Oceanography*, 20(2), pp. 28–33. doi: 10.5670/oceanog.2007.45.

Posten, C. and Rosello-Sastre, R. (2011) 'Microalgae Reactors', in *Ullmann's Encyclopedia of Industrial Chemistry*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA. doi: 10.1002/14356007.o16_o01.

Le Quéré, C. *et al.* (2018) 'Global Carbon Budget 2017', *Earth Syst. Sci. Data Etsushi Kato Markus Kautz Ralph F. Keeling Kees Klein Goldewijk Nathalie Lefèvre Andrew Lenton Danica Lombardozzi Nicolas Metzl Yukihiro Nojiri Antonio Padin Janet Reimer*, 101033373915134830(10), pp. 405–448. doi: 10.5194/essd-10-405-2018.

Raghukumar, C. *et al.* (2001) ‘Degradation of crude oil by marine cyanobacteria’, *Appl Microbiol Biotechnol*, 57, pp. 433–436. Available at: file:///Users/afiqdurr/Downloads/Degradation_of_crude_oil_by_ma.pdf (Accessed: 4 August 2018).

Ramanan, R. *et al.* (2016) ‘Algae–bacteria interactions: Evolution, ecology and emerging applications’. doi: 10.1016/j.biotechadv.2015.12.003.

Ratti, S., Giordano, M. and Morse, D. (2007) ‘CO2 -Concentrating Mechanisms Of The Potentially Toxic Dinoflagellate Protoceratum Reticulatum (Dinophyceae, Gonyaulacales)’, *Journal of Phycology*. John Wiley & Sons, Ltd (10.1111), 43(4), pp. 693–701. doi: 10.1111/j.1529-8817.2007.00368.x.

Read, B. A. *et al.* (2013) ‘Pan genome of the phytoplankton Emiliania underpins its global distribution’, *Nature*. Nature Publishing Group, 499(7457), pp. 209–213. doi: 10.1038/nature12221.

Reynolds, C., S. (2006) *Ecology of Phytoplankton*. Cambridge University Press.

Riebesell, U. *et al.* (2000) ‘Reduced calcification of marine plankton in response to increased atmospheric CO2.’, *Nature*, 407(6802), pp. 364–7. doi: 10.1038/35030078.

Riebesell, U. (2004) ‘Effects of CO2 Enrichment on Marine Phytoplankton’, *Journal of Oceanography*. Kluwer Academic Publishers-Plenum Publishers, 60(4), pp. 719–729. doi: 10.1007/s10872-004-5764-z.

Riebesell, U. *et al.* (2008) ‘Comment on “Phytoplankton Calcification in a High-CO2 World”’, *Science*, 322(5907), p. 1466b–1466b. doi: 10.1126/science.1161096.
Riebesell, U. *et al.* (2011) *Guide to best practices for ocean acidification research and data processing*. Edited by U. Riebesell et al. doi: EU24328EN.

Riebesell, U. *et al.* (2013) ‘Arctic ocean acidification: pelagic ecosystem and biogeochemical responses during a mesocosm study &quot’;, *Biogeosciences*, 10, pp. 5619–5626. Available at: www.biogeosciences.net/10/5619/2013/ (Accessed: 5 April

2018).

Riebesell, U. *et al.* (2013) ‘Technical Note: A mobile sea-going mesocosm system – new opportunities for ocean change research’, *Biogeosciences*, 10(3), pp. 1835–1847. doi: 10.5194/bg-10-1835-2013.

Robbins, L. L., Hansen, M. E. and Meylan, S. C. (2016) *CO2calc: A User-Friendly Seawater Carbon Calculator for Windows, Mac OS X, and iOS (iPhone)*. Available at: https://pubs.usgs.gov/of/2010/1280/pdf/ofr_2010-1280.pdf (Accessed: 28 May 2018).

Rodrigues, D. F. *et al.* (2009) ‘Biogeography of two cold-adapted genera: Psychrobacter and Exiguobacterium’, *The ISME Journal*. Nature Publishing Group, 3(6), pp. 658–665. doi: 10.1038/ismej.2009.25.

Roeselers, G. *et al.* (2006) ‘On the reproducibility of microcosm experiments - different community composition in parallel phototrophic biofilm microcosms’. doi: 10.1111/j.1574-6941.2006.00172.x.

Rokitta, S. D. *et al.* (2014) ‘Emiliania huxleyi endures N-limitation with an efficient metabolic budgeting and effective ATP synthesis.’, *BMC genomics*. BioMed Central, 15(1), p. 1051. doi: 10.1186/1471-2164-15-1051.

Rokitta, S. D. and Rost, B. (2012) ‘Effects of CO2 and their modulation by light in the life-cycle stages of the coccolithophore Emiliania huxleyi’, *Limnology and Oceanography*. John Wiley & Sons, Ltd, 57(2), pp. 607–618. doi: 10.4319/lo.2012.57.2.0607.

Roy, A.-S. *et al.* (2013) ‘Ocean acidification shows negligible impacts on high-latitude bacterial community structure in coastal pelagic mesocosms’, *Biogeosciences*, 10(1), pp. 555–566. doi: 10.5194/bg-10-555-2013.

Sakshaug, E. and Holm-Hansen, O. (1977) ‘Chemical composition of Skeletonema costatum (Grev.) Cleve And Pavlova (monochrysis) Lutheri (droop) green as a function of nitrate-, phosphate-, and iron-limited growth’, *Journal of Experimental Marine Biology and Ecology*, 29(1), pp. 1–34. doi: 10.1016/0022-0981(77)90118-6.

Sargian, P. *et al.* (2007) ‘Multiple stressors on an Antarctic microplankton assemblage: water soluble crude oil and enhanced UVBR level at Ushuaia (Argentina)’, *Polar Biology*. Springer-Verlag, 30(7), pp. 829–841. doi: 10.1007/s00300-006-0243-1.

Schloss, P. D. *et al.* (2009) ‘Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities.’, *Applied and environmental microbiology*. American Society for Microbiology, 75(23), pp. 7537–41. doi: 10.1128/AEM.01541-09.

Schneiker, S. *et al.* (2006) ‘Genome sequence of the ubiquitous hydrocarbon-degrading marine bacterium Alcanivorax borkumensis’, *Nature Biotechnology*, 24(8), pp. 997–1004. doi: 10.1038/nbt1232.

Schoffman, H. *et al.* (2016) ‘Iron-Nutrient Interactions within Phytoplankton.’, *Frontiers in plant science*. Frontiers Media SA, 7, p. 1223. doi: 10.3389/fpls.2016.01223.

Schopf, J. W. (2000) ‘The Fossil Record: Tracing the Roots of the Cyanobacterial Lineage’, in *The Ecology of Cyanobacteria*. Dordrecht: Kluwer Academic Publishers, pp. 13–35. doi: 10.1007/0-306-46855-7_2.

Segev, E. *et al.* (2016) ‘Bacterial influence on alkenones in live microalgae’, *Journal of Phycology*, 52(1), pp. 125–130. doi: 10.1111/jpy.12370.

Seyedsayamdost, M. R., Carr, G., *et al.* (2011) ‘Roseobacticides: Small Molecule Modulators of an Algal-Bacterial Symbiosis’, *Journal of the American Chemical Society*. American Chemical Society, 133(45), pp. 18343–18349. doi: 10.1021/ja207172s.

Seyedsayamdost, M. R., Case, R. J., *et al.* (2011) ‘The Jekyll-and-Hyde chemistry of Phaeobacter gallaeciensis’, *Nature Chemistry*. Nature Publishing Group, 3(4), pp. 331–335. doi: 10.1038/nchem.1002.

Seymour, J. R. *et al.* (2017) ‘Zooming in on the phycosphere: The ecological interface

for phytoplankton-bacteria relationships', *Nature Microbiology*. doi: 10.1038/nmicrobiol.2017.65.

Sharma, A. and Thakur, I. S. (2009) 'Identification and characterization of integron mediated antibiotic resistance in pentachlorophenol degrading bacterium isolated from the chemostat', *Journal of Environmental Sciences*. Elsevier, 21(6), pp. 858–864. doi: 10.1016/S1001-0742(08)62353-0.

Shaw, S. L., Chisholm, S. W. and Prinn, R. G. (2003) 'Isoprene production by Prochlorococcus, a marine cyanobacterium, and other phytoplankton', *Marine Chemistry*, 80(4), pp. 227–245. doi: 10.1016/S0304-4203(02)00101-9.

Shete, A. M. *et al.* (2015) *Isolation, Biotyping, Biochemical and Physiological Characterization of Marine Acinetobacter Isolated from West Coast of India*, *Int.J.Curr.Microbiol.App.Sci*. Available at: http://www.ijcmas.com (Accessed: 24 August 2018).

Shi, D. *et al.* (2012) 'Ocean acidification slows nitrogen fixation and growth in the dominant diazotroph Trichodesmium under low-iron conditions', *PNAS*, 109(45), pp. E3094–E3100. doi: 10.1073/pnas.1216012109.

Shi, D., Xu, Y. and Morel, F. M. M. (2009) 'Effects of the pH/pCO2 control method on medium chemistry and phytoplankton growth', *Biogeosciences*, 6(7), pp. 1199–1207. doi: 10.5194/bg-6-1199-2009.

Sikkema, J. *et al.* (1995) 'Mechanisms of Membrane Toxicity of Hydrocarbons', *MICROBIOLOGICAL REVIEWS*, 59(2), pp. 201–222. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC239360/pdf/590201.pdf (Accessed: 5 July 2018).

Siron, R. *et al.* (1991) 'Water-soluble petroleum compounds: chemical aspects and effects on the growth of microalgae', *Science of The Total Environment*. Elsevier, 104(3), pp. 211–227. doi: 10.1016/0048-9697(91)90073-N.

Smith, D., F. *et al.* (2018) '21 Tesla FT-ICR Mass Spectrometer for Ultrahigh-

Resolution Analysis of Complex Organic Mixtures', *Analytical Chemistry*, 90, pp. 2041–2047. Available at: https://pubs.acs.org/doi/pdf/10.1021/acs.analchem.7b04159 (Accessed: 26 June 2018).

Smith, D. J. *et al.* (2009) 'Survivability of Psychrobacter cryohalolentis K5 Under Simulated Martian Surface Conditions', *Astrobiology*, 9(2), pp. 221–228. doi: 10.1089/ast.2007.0231.

Smith, R. E. H. and Kalff, J. (1982) 'Size-dependent phosphorus uptake kinetics and cell quota in phytoplankton', *Journal of Phycology*. Wiley/Blackwell (10.1111), 18(2), pp. 275–284. doi: 10.1111/j.1529-8817.1982.tb03184.x.

Sobrino, C., Ward, M. L. and Neale, P. J. (2008) 'Acclimation to elevated carbon dioxide and ultraviolet radiation in the diatom Thalassiosira pseudonana : Effects on growth, photosynthesis, and spectral sensitivity of photoinhibition', *Limnology and Oceanography*. John Wiley & Sons, Ltd, 53(2), pp. 494–505. doi: 10.4319/lo.2008.53.2.0494.

Solovchenko, A. and Khozin-Goldberg, I. (2013) 'High-CO 2 tolerance in microalgae: possible mechanisms and implications for biotechnology and bioremediation', *Biotechnology Letter*, 35, pp. 1745–1752. doi: 10.1007/s10529-013-1274-7.

Sorigué, D. *et al.* (2016) 'Microalgae Synthesize Hydrocarbons from Long-Chain Fatty Acids via a Light-Dependent Pathway.', *Plant physiology*. American Society of Plant Biologists, 171(4), pp. 2393–405. doi: 10.1104/pp.16.00462.

Soto, C. *et al.* (1975) 'Effect of naphthalene and aqueous crude oil extracts on the green flagellate Chlamydomonas angulosa. I. Growth', *Canadian Journal of Botany*. NRC Research Press Ottawa, Canada, 53(2), pp. 109–117. doi: 10.1139/b75-017.

Spetland, F. *et al.* (2007) 'Sexual reproduction of Geodia barretti (Bowerbank, 1858)(Porifera, Astrophorida) in two Scandinavian fjords', *Porifera Research: Biodiversity, Innovation and Sustainability*. Available at: file:///Users/afiqdurr/Downloads/Sexual_reproduction_of_Geodia_barretti_Bowerbank_1.pdf (Accessed: 5 November 2018).

Spielmeyer, A. and Pohnert, G. (2012) ‘Influence of temperature and elevated carbon dioxide on the production of dimethylsulfoniopropionate and glycine betaine by marine phytoplankton’, *Marine Environmental Research*. Elsevier, 73, pp. 62–69. doi: 10.1016/J.MARENVRES.2011.11.002.

Spilimbergo, S. *et al.* (2005) ‘Determination of extracellular and intracellular pH ofBacillus subtilis suspension under CO2 treatment’, *Biotechnology and Bioengineering*. Wiley-Blackwell, 92(4), pp. 447–451. doi: 10.1002/bit.20606.

Spungin, D., Berman-Frank, I. and Levitan, O. (2014) ‘Trichodesmium’s strategies to alleviate phosphorus limitation in the future acidified oceans.’, *Environmental microbiology*, 16(6), pp. 1935–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25009839 (Accessed: 31 May 2019).

Stanbury, P. F., Whitaker, A. and Hall, S. J. (1995) ‘Microbial Growth Kinetics’, in *Principles of Fermentation Technology*. 2nd Editio. MPG Books Ltd, pp. 13–34.

Suja, L. D., Summers, S. and Gutierrez, T. (2017) ‘Role of EPS, Dispersant and Nutrients on the Microbial Response and MOS Formation in the Subarctic Northeast Atlantic’, *Frontiers in Microbiology*. Frontiers, 8, p. 676. doi: 10.3389/fmicb.2017.00676.

Sun, J. *et al.* (2011) ‘One Carbon Metabolism in SAR11 Pelagic Marine Bacteria’, *PLoS ONE*. Edited by J. A. Gilbert. Public Library of Science, 6(8), p. e23973. doi: 10.1371/journal.pone.0023973.

Sundby, S., Drinkwater, K. F. and Kjesbu, O. S. (2016) ‘The North Atlantic Spring-Bloom System—Where the Changing Climate Meets the Winter Dark’, *Frontiers in Marine Science*. Frontiers, 3, p. 28. doi: 10.3389/fmars.2016.00028.

Suttle, C. A. *et al.* (1986) *Short Communication Grazing of planktonic diatoms by microflagellates, Journal of Plankton Research*. Available at: https://academic.oup.com/plankt/article-abstract/8/2/393/1441960 (Accessed: 25 July 2018).

Swan, B. K. *et al.* (2013) ‘Prevalent genome streamlining and latitudinal divergence of planktonic bacteria in the surface ocean.’, *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 110(28), pp. 11463–8. doi: 10.1073/pnas.1304246110.

Tatters, A. O. *et al.* (2013) ‘High CO2 promotes the production of paralytic shellfish poisoning toxins by Alexandrium catenella from Southern California waters’, *Harmful Algae*. Elsevier, 30, pp. 37–43. doi: 10.1016/J.HAL.2013.08.007.

Techtmann, S. M. *et al.* (2016) ‘Colwellia psychrerythraea Strains from Distant Deep Sea Basins Show Adaptation to Local Conditions’, *Frontiers in Environmental Science*. Frontiers, 4, p. 33. doi: 10.3389/fenvs.2016.00033.

Teeling, H. *et al.* (2012) ‘Substrate-Controlled Succession of Marine Bacterioplankton Populations Induced by a Phytoplankton Bloom’, *Science*, 336(6081), pp. 608–611. doi: 10.1126/science.1218344.

Thiel, V. *et al.* (2010) ‘Identification and biosynthesis of tropone derivatives and sulfur volatiles produced by bacteria of the marine Roseobacter clade.’, *Organic & biomolecular chemistry*, 8(1), pp. 234–46. doi: 10.1039/b909133e.

Thompson, H. *et al.* (2017) ‘Enhanced crude oil biodegradative potential of natural phytoplankton-associated hydrocarbonoclastic bacteria’, *Environmental Microbiology*, 19(7), pp. 2843–2861. doi: 10.1111/1462-2920.13811.

Thornber, C. S. (2006) ‘Functional properties of the isomorphic biphasic algal life cycle’, *Integrative and Comparative Biology*. Oxford University Press, 46(5), pp. 605–614. doi: 10.1093/icb/icl018.

Tillett, D. and Neilan, B. A. (2000) ‘Xanthogenate nucleic acid isolation from cultured and environmental cyanobacteria’, *Journal of Phycology*. Blackwell Science Inc, 36(1), pp. 251–258. doi: 10.1046/j.1529-8817.2000.99079.x.

Tilstone, G. *et al.* (2016) ‘Effect of CO2 enrichment on phytoplankton photosynthesis

in the North Atlantic sub-tropical gyre', *Progress in Oceanography*. doi: 10.1016/j.pocean.2016.12.005.

van Tol, H. M., Amin, S. A. and Armbrust, E. V. (2017) 'Ubiquitous marine bacterium inhibits diatom cell division.', *The ISME journal*. Nature Publishing Group, 11(1), pp. 31–42. doi: 10.1038/ismej.2016.112.

Tortell, P. D. *et al.* (1999) 'Marine bacteria and biogeochemical cycling of iron in the oceans', *FEMS Microbiology Ecology*. Wiley/Blackwell (10.1111), 29(1), pp. 1–11. doi: 10.1111/j.1574-6941.1999.tb00593.x.

Tortell, P. D. *et al.* (2002) 'CO2 effects on taxonomic composition and nutrient utilization in an Equatorial Pacific phytoplankton assemblage', *Marine Ecology Progress Series*, 236, pp. 37–43. doi: 10.3354/meps236037.

Tortell, P. D. *et al.* (2008) 'CO2 sensitivity of Southern Ocean phytoplankton', *Geophysical Research Letters*. John Wiley & Sons, Ltd, 35(4), p. L04605. doi: 10.1029/2007GL032583.

Trevors, J. T. (1998) 'Bacterial Biodiversity in Soil with an Emphasis on Chemically-Contaminated Soils', *Water, Air, and Soil Pollution*. Kluwer Academic Publishers, 101(1/4), pp. 45–67. doi: 10.1023/A:1004953404594.

Tringe, S. G. and Rubin, E. M. (2005) 'Metagenomics: DNA sequencing of environmental samples', *Nature Reviews Genetics*. Nature Publishing Group, 6(11), pp. 805–814. doi: 10.1038/nrg1709.

Tsuji, Y. *et al.* (2015) 'Quantitative Analysis of Carbon Flow into Photosynthetic Products Functioning as Carbon Storage in the Marine Coccolithophore, Emiliania huxleyi', *Marine Biotechnology*. Springer, 17(4), pp. 428–440. doi: 10.1007/s10126-015-9632-1.

Tukaj, Z., Bohdanowicz, J. and Aksmann, A. (1998) 'A morphometric and stereological analysis of ultrastructural changes in two Scenedesmus (Chlorococcales, Chlorophyta) strains subjected to diesel fuel oil pollution', *Phycologia*. The International

Phycological Society Phycologia Business Office, Allen Press, 810 East 10th Street, P.O. Box 1897, Lawrence, KS 66044-8897 , 37(5), pp. 388–393. doi: 10.2216/i0031-8884-37-5-388.1.

Tyrrell, T. and Merico, A. (2004) 'Emiliania huxleyi: bloom observations and the conditions that induce them', in *Coccolithophores*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 75–97. doi: 10.1007/978-3-662-06278-4_4.

Uchimiya, M. *et al.* (2016) 'Coupled Response of Bacterial Production to a Wind-Induced Fall Phytoplankton Bloom and Sediment Resuspension in the Chukchi Sea Shelf, Western Arctic Ocean', *Frontiers in Marine Science*. Frontiers, 3, p. 231. doi: 10.3389/fmars.2016.00231.

Valentine, D. L. *et al.* (2014) 'Fallout plume of submerged oil from Deepwater Horizon.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 111(45), pp. 15906–11. doi: 10.1073/pnas.1414873111.

Valero, M. *et al.* (1992) 'Evolution of Alternation of Haplois and Diploid Phases in Life Cycles', *Trends in Ecology & Evolution*, 7(1), pp. 25–29.

Vargo, G. ., Hutchins, M. and Almquist, G. (1982) 'The effect of low, chronic levels of no. 2 fuel oil on natural phytoplankton assemblages in microcosms: 1. Species composition and seasonal succession', *Marine Environmental Research*. Elsevier, 6(4), pp. 245–264. doi: 10.1016/0141-1136(82)90040-X.

Vásquez, M. *et al.* (2002) *Limited selection of sodium channel blocking toxin-producing bacteria from paralytic shellfish toxin-contaminated mussels (Aulacomya ater), Research in Microbiology*. Available at: www.elsevier.com/locate/resmic (Accessed: 3 August 2018).

Venter, J. C. *et al.* (2004) 'Environmental Genome Shotgun Sequencing of the Sargasso Sea', *Science*, 304(5667), pp. 66–74. doi: 10.1126/science.1093857.

Vetter, W. (1994) *F. W. McLafferty, F. Turecek. Interpretation of mass spectra. Fourth*

edition (1993). University Science Books, Mill Valley, California, Biological Mass Spectrometry. Wiley-Blackwell. doi: 10.1002/bms.1200230614.

Vila, J. *et al.* (2010) 'Microbial community structure of a heavy fuel oil-degrading marine consortium: Linking microbial dynamics with polycyclic aromatic hydrocarbon utilization', *FEMS Microbiology Ecology*, 73(2), pp. 349–362. doi: 10.1111/j.1574-6941.2010.00902.x.

Voss, M. *et al.* (2013) 'The marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of climate change', *Philosophical Transactions of the Royal Society B*. doi: 10.1098/rstb.2013.0121.

Wang, H. *et al.* (2015) 'Identification of Genetic Modules Mediating the Jekyll and Hyde Interaction of Dinoroseobacter shibae with the Dinoflagellate Prorocentrum minimum', *Frontiers in Microbiology*, 6, p. 1262. doi: 10.3389/fmicb.2015.01262.

Ward, B. B. and Van Oostende, N. (2016) 'Phytoplankton assemblage during the North Atlantic spring bloom assessed from functional gene analysis', *Journal of Plankton Research*. Oxford University Press, 38(5), pp. 1135–1150. doi: 10.1093/plankt/fbw043.

Weinbauer, M. G., Mari, X. and Gattuso, J.-P. (2011) 'Effect of ocean acidification on the diversity and activity of heterotrophic marine microorganisms', *Ocean acidification*, (October 2015), pp. 83–98.

Weinbauer, M. G. and Wenderoth, D. F. (2008) 'Microbial Diversity and Ecosystem Functions - the Unmined Riches', *Electronic Journal of Biotechnology*, 5(April 2002), pp. 1–7. doi: 10.2225/vol5-issue1-fulltext-11.

Welschmeyer, N. A. (1994) 'Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments', *Limnology and Oceanography*. Wiley-Blackwell, 39(8), pp. 1985–1992. doi: 10.4319/lo.1994.39.8.1985.

Whitney, S. M., Shaw, D. C. and Yellowlees, D. (1995) 'Evidence that some dinoflagellates contain a ribulose-1,5-bisphosphate carboxylase / oxygenase related to that of the α-proteobacteria', *Proceedings of the Royal Society of London. Series B:*

Biological Sciences, 259(1356), pp. 271–275. doi: 10.1098/rspb.1995.0040.

Whitton, B. A. and Potts, M. (no date) 'Introduction to the Cyanobacteria', in *The Ecology of Cyanobacteria*. Dordrecht: Kluwer Academic Publishers, pp. 1–11. doi: 10.1007/0-306-46855-7_1.

Windler, M. *et al.* (2014) 'Influence of bacteria on cell size development and morphology of cultivated diatoms', *Phycological Research*. Wiley/Blackwell (10.1111), 62(4), pp. 269–281. doi: 10.1111/pre.12059.

Witt, V. *et al.* (2011) 'Effects of ocean acidification on microbial community composition of, and oxygen fluxes through, biofilms from the Great Barrier Reef', *Environmental Microbiology*. Wiley/Blackwell (10.1111), 13(11), pp. 2976–2989. doi: 10.1111/j.1462-2920.2011.02571.x.

Wolf-Gladrow, D., A. *et al.* (1999) 'Direct effects of CO2 concentration on growth and isotopic composition of marine plankton', *Tellus B*. Wiley/Blackwell (10.1111), 51(2), pp. 461–476. doi: 10.1034/j.1600-0889.1999.00023.x.

Wu, X. *et al.* (2012) 'Growth and photosynthesis of a diatom grown under elevated CO2 in the presence of solar UV radiation', *Fundamental and Applied Limnology / Archiv für Hydrobiologie*. Schweizerbart'sche Verlagsbuchhandlung, 180(4), pp. 279–290. doi: 10.1127/1863-9135/2012/0299.

Wu, Y., Gao, K. and Riebesell, U. (2010) 'CO2-induced seawater acidification affects physiological performance of the marine diatom Phaeodactylum tricornutum', *Biogeosciences*, 7, pp. 2915–2923. doi: 10.5194/bg-7-2915-2010.

Xing, P. *et al.* (2015) 'Niches of two polysaccharide-degrading Polaribacter isolates from the North Sea during a spring diatom bloom.', *The ISME journal*. Nature Publishing Group, 9(6), pp. 1410–22. doi: 10.1038/ismej.2014.225.

Xiong, S. *et al.* (2015) 'Crude oil degradation by bacterial consortia under four different redox and temperature conditions', *Applied Microbiology and Biotechnology*, 99(3), pp. 1451–1461. doi: 10.1007/s00253-014-6042-7.

Yakimov, M. M. *et al.* (2003) ‘Oleispira antarctica gen. nov., sp. nov., a novel hydrocarbonoclastic marine bacterium isolated from Antarctic coastal sea water’, *International Journal of Systematic and Evolutionary Microbiology*, 53(3), pp. 779–785. doi: 10.1099/ijs.0.02366-0.

Yakimov, M. M., Timmis, K. N. and Golyshin, P. N. (2007) ‘Obligate oil-degrading marine bacteria’, *Current Opinion in Biotechnology*, 18(3), pp. 257–266. doi: 10.1016/j.copbio.2007.04.006.

Yamane, K. *et al.* (2013) ‘Pyrolytic generation of petroleum crude oils from the marine phytomicroalgal coccolithophore Emiliania huxleyi (haptophyta) and preparation of n-alkane-rich biofuel’, *Energy and Fuels*. American Chemical Society, 27(12), pp. 7470–7479. doi: 10.1021/ef401618t.

Yang, T. *et al.* (2016) ‘Pulsed blooms and persistent oil-degrading bacterial populations in the water column during and after the Deepwater Horizon blowout’, *Deep Sea Research Part II: Topical Studies in Oceanography*. Pergamon, 129, pp. 282–291. doi: 10.1016/J.DSR2.2014.01.014.

Zeng, X., Chen, X. and Zhuang, J. (2015) ‘The positive relationship between ocean acidification and pollution’, *Marine Pollution Bulletin*. Pergamon, 91(1), pp. 14–21. doi: 10.1016/J.Marpolbul.2014.12.001.

Zhang, D.-C. *et al.* (2008) ‘Colwellia polaris sp. nov., a psychrotolerant bacterium isolated from Arctic sea ice’, *International Journal of Systematic and Evolutionary Microbiology*. Microbiology Society, 58(8), pp. 1931–1934. doi: 10.1099/ijs.0.65596-0.

Zhang, R. *et al.* (2012) ‘Response of bacterioplankton community structure to an artificial gradient of <i>p</i>CO₂ in the Arctic Ocean’, *Biogeosciences Discussions*, 9(8), pp. 10645–10668. doi: 10.5194/bgd-9-10645-2012.

Zondervan, I., Rost, B. and Riebesell, U. (2002) ‘Effect of CO2 concentration on the PIC/POC ratio in the coccolithophore Emiliania huxleyi grown under light-limiting

conditions and different daylengths', *Journal of Experimental Marine Biology and Ecology*. Elsevier, 272(1), pp. 55–70. doi: 10.1016/S0022-0981(02)00037-0.